LABORATORY DIRECTIONS

IN

GENERAL ZOOLOGY

By

Winterton C. Curtis

Professor of Zoology, University of Missouri

and

Gideon S. Dodds

Asst. Professor of Histology and Embryology,
West Virginia University

AUGUST, 1919

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PREFACE

The course in General Zoology, of which the work outlined by these directions constitutes a part, is in marked contrast to the "phylum" course, in which an extensive series of forms is examined, mainly with reference to their structural peculiarities. In the phylum course, each animal is considered, primarily, as a representative of the group to which it belongs. Its general biological aspects are regarded as incidental. Such a course is demanded in the later curriculum of students who go on in zoology. But we maintain that a course which consists principally of a survey of the animal kingdom, phylum by phylum, is not suited to the needs of such students as are destined for no further contact with zoological departments. It is often forgotten that the general course is not an introduction to zoology, in the sense that the student will be expected to pursue the acquaintance. In the vast majority of cases, it is part of an introduction to human life. We are teaching future citizens and not future zoologists. Even where the student has some professional motivation, such as an interest in medicine or agriculture, the best introduction is a course taught from this "humanistic" standpoint. The humanistic ideal is the ideal of that which is worth while for man. Humanistic teaching is teaching in such a manner that the student "feels" the worthwhileness of the subject matter. It is an attempt to create appreciation. Such an emphasis is far removed from the "economic" emphasis which attempts to stress the bread-and-butter facts, although these facts are part of the worthwhileness of the whole. Zoological science is filled to the brim with facts of human interest. When we teach zoology to human beings, who are destined never again to have contact with the zoological classroom, we should teach the zoological phenomena which bear the most intimate relation

to human life. While not maintaining that the phylum course is incapable of accomplishing this result, we believe that such a course is, at best, only an indirect approach to the zoological subject matter which should be regarded as most important.

With this humanistic point of view, the laboratory work of the course might be organized in a variety of ways. It might consist in the study of structures and functions, without much reference to the consideration of animals as organized units. It might consist of the intensive study of a single animal. The objection to the first of these plans is that it tends to weaken the conception of the animal as a unified individual. There is danger that the study of a piece at a time or a function at a time will obliterate the picture of the unified whole. The objection to the intensive study of a single animal is that no single form illustrates all the interesting biological phenomena which should find a place in the course in General Zoology. In our opinion, the must desirable plan is the study of a limited number of forms which are particularly suited to illustrate the fundamental biological phenomena. Upon such a plan, these laboratory directions have been developed.

The frog is chosen as the introductory type, because of its availability; and because it is, like man, a vertebrate. The use of a vertebrate enables the student to utilize, at the outset, the knowledge he may have concerning the structures and functions of his own body; and to further extend his knowledge by an application of the facts learned from the frog. In addition to their human application, the facts thus discovered become the observational basis for a study of the fundamental activities and functions of the animal body. Then follows a study of a limited number of unicellular forms, during which the student examines the various functions as performed by animals of minute size and essentially without organs. He thus becomes acquainted with biological phenomena reduced to their lowest terms; and learns that a given organ is not essential to the performance of a given function. Other fundamental phenomena, which are well illustrated by the protozoa, are considered in this connection. Following this comes

a study of the hydra, during which the relation between unicellular and multicellular forms receives consideration. This is followed by the study of one or more multicellular animals of greater complexity (earthworm, crayfish, or mussel), which shows the performance of the now familiar functions by organs of quite different type and adapted to a different environment. The grasshopper and other insects present examples of animals specialized in directions which are especially useful in teaching the facts of adaptation, behavior, and life-histories. The flatworms may be used in illustration of parasitism. The development of the frog, supplemented by that of the starfish and chick, constitutes the foundation for a discussion of reproduction. The subject of genetics, including human evolution, is considered without accompanying laboratory study. Thus at the close we come again to the intensely human aspects of zoology.

The textbook used by the authors of this manual, during their work together at the University of Missouri, was Parker and Parker's, "Practical Zoology". This text was regarded as inadequate, because so exclusively morphological in character. We know of no single volume which approximates the body of subject matter and the arrangement we would choose for this course. The senior author is now making a trial of an elementary book on human physiology and a textbook of zoology. For the year 1919-20, he has chosen the "Elements of Physiology", by Hough and Sedgwick; and the "General Zoology", by A. S. Pearse. The text on physiology furnishes subject matter which may be readily correlated with the laboratory work upon the frog and with the subject matter of the earlier lectures. The volume by Pearse, although presenting a phylum course developed from the ecological standpoint, emphasizes many of the humanistic aspects of zoology which we have featured in the past. Such a combination of texts we regard as workable although not ideal.

It will be observed that the directions in this manual are given in a somewhat detailed and specific fashion. We have, in general, avoided the non-sentence style, in the belief that

college students need the maximum contact with the well-formed sentence, as a part of their education. In our experience, freshmen and sophmores should have the training that goes with precision of direction and organization. We even maintain that there is something to be said for mere "learning to follow directions", if the student has never been trained to careful reading in matters of fact. On the side of technical procedure, the beginner must have rather complete direction. On the side of the interpretation of facts, the directions should be precise and definite, with avoidance of both cryptic statement and unnecessary explanation. As the present manual is the outcome of earlier printed editions, we have thoroughly tested its form and phraseology by use in our classes.

A word may be added regarding the sources of these directions. The present publication is a revision, made jointly by the two authors, of the "Laboratory Directions" published in 1913 by the senior author. It is impossible to acknowledge individually the many friendly criticisms and suggestions which have furnished material for the present work. This pamphlet is a temporary crystallization of the laboratory instruction in General Zoology at the University of Missouri. We are indebted to all our colleagues and assistants, with whom the problems of the course have been a frequent subject of discussion. We gratefully acknowledge the obligation.

WINTERTON C. CURTIS

GIDEON S. DODDS

August, 1919

CONTENTS.

THE FROG

PHYLUM, CHORDATA. SUB-PHYLUM, VERTEBRATA.
CLASS, AMPHIBIA

I. EXTERNAL FEATURES

Exercise 1. General Structure and Activities

(a) The following directions apply particularly to the "leopard frog", **Rana pipiens.** They may, however, be used for any of the common species. Examine a preserved frog or one recently killed. Recognize **anterior** and **posterior** ends, **dorsal** and **ventral** surfaces, right and left sides. Notice the soft, slimy nature of the **skin** and the absence of hair, feathers, or scales. What is the relation of such a skin to the habitat of the frog? Is the frog adapted to life in dry places? Notice the distribution of color on the skin. Are the right and left sides colored alike; dorsal and ventral surfaces? Do you know of any other animals that are lighter colored underneath? Why? Is the head of the frog sharply separated from the body? What is the use of the long, flexible neck in birds and mammals? What peculiar structure has the elephant to compensate for its short neck? Notice the **mouth,** the **anus,** the **nostrils,** and the **eyes.** Compare the **eyelids** with those of the human eye. Just posterior to the eye is the **tympanic membrane** or ear-drum. Compare the ear of the frog with your own. Notice that fore and hind limbs have the same general structure: that is, **upper arm, forearm, wrist,** and **hand,** corresponding to **thigh, shin, ankle,** and **foot.** Notice how this similar fundamental structure is modified for quite different purposes in the two pairs of limbs. Compare with the limbs of a human being.

(b) Study living frogs in water and on a solid surface. How is each pair of limbs adapted and used for locomotion (1) on land, (2) in water? Which of these modes of locomotion seems to be the primary one for the frog? Give your reasons. Compare structure and uses of the limbs in dog, ape, duck, and hen. Observe position of frog as it "floats" in the water. Is it a good position for a quick retreat to the bottom? Observe just how the frog makes this retreat when frightened. What is the position of the eyes and nostrils when the animal is floating? What is the use of this? Is the resting position of the frog when out of water such as to enable it to make a quick escape from danger? On what must the frog depend for protection from other animals? Compare in this respect with deer, horse, bear, turtle, rattlesnake, and man. Is the frog well or poorly protected? Make a list of some of the more common means of protection which animals possess.

(c) Place the animal in the dissecting pan dorsal side up, pinning out the fore and hind limbs with digits well spread. Have pencils carefully sharpened. Rule a faint line lengthwise in the middle of a sheet of drawing paper and, using this to represent the median plane of the animal, show the **bilateral symmetry.** Draw on a scale of 1 as thus seen from the dorsal aspect. Measure all the distances accurately with ruler or compasses; and show all the parts, to which your attention has been called in the foregoing section, so far as they appear in this view. Finish the drawing in simple outlines, without shading. Label thoroughly, making label lines with a ruler and arranging neatly the lines and the names to which they lead. The scale of a drawing is commonly indicated thus: x ½, x 1, x 2, etc., as the case may be.

II. THE MOUTH

Exercise 2. The Mouth Cavity

(a) Open the mouth wide, cutting a little at the corners if necessary. Locate the **teeth** by rubbing with a finger. Are teeth found on both jaws? Which way do the teeth slant?

Examine with handlens. Are they useful in chewing as are your own teeth? What is their use? Observe two patches of teeth on roof of the mouth, the **vomerine** teeth, so called because on a bone called the vomer. Close to the vomerine teeth are the **internal nostrils.** Pass a bristle through one nostril. Notice the swellings made by the eyes, and just back of them the **Eustachian tubes,** leading to the middle ears. Posteriorly, the mouth narrows into the **œsophagus** which leads to the stomach. Explore with handle of needle. Just ventral to the œsophagus is a raised area perforated by a vertical slit, the **glottis,** through which air enters the lungs. What is the course of air from the exterior to the lungs? Compare with the human body in this respect. Why is it that a man can breathe through either mouth or nose while the frog cannot breathe at all with its mouth open? How does the place of attachment of the tongue differ from that in man? How does the frog use its tongue? Make a drawing (x 2), showing the widely opened mouth as seen from in front.

III. THE BODY CAVITY AND ITS ORGANS

Exercise 3. The Body Wall

(a) Fasten the frog, ventral side up, in a dissecting pan, by means of a pin thrust obliquely through each arm. With scissors, cut through the skin, but not the underlying muscles, along the mid-ventral line, from tip of jaw to posterior end of body. The skin is attached to the muscles only at certain places, and the space between contains a colorless fluid called **lymph.** Cut the skin outward, at right angles from the first cut, in the region of the arms and again at the posterior end of the body; pin back the flaps. Notice the **blood vessels** of the skin; **veins,** conspicuous and dark colored; **arteries,** inconspicuous and usually colorless. Why this difference? Examine with a lens. In a similar manner, arteries and veins are distributed throughout the body. Notice the muscles of the body wall.

(b) Cut through the muscle layer, a little to one side of

the median line, exposing the **body cavity** or **cœlome.** By cutting and reflecting the muscle layer, as with the skin, expose the **viscera.** In the region of the arms it will be necessary to cut through certain bones, the arrangement of which may be seen by reference to a prepared skeleton. In the following study of the viscera, the organs may be displaced and pushed aside as necessary, but **not cut or removed** until specific directions are given. In identifying the parts, work with care and do not injure organs that must be studied later.

(c) If the animal is a female, the most conspicuous organs are likely to be the **ovaries,** containing many black-and-white eggs, which may largely fill the body cavity and obscure other organs. If you have a male animal, no ovaries are present, and the digestive organs are more prominent. Determine the sex of your specimen; and in the following pages follow only those parts which apply to this sex.

(d) If you have a male, omit this paragraph and go at once to the study of the digestive organs (Exercise 4). If you have a female, remove both ovaries as follows: Push aside and separate the right and left ovaries, and discover their shape and place of attachment. Now pick out both ovaries, bit by bit with your forceps, being careful not to injure the other organs. Estimate the total number of eggs. The large black-and-white eggs are those which will be laid at the next breeding season. Can you discover smaller eggs by using the handlens? If every one of these eggs should live and produce a full grown frog, what about the rapidity with which the world would become stocked with frogs? In the female there are two oviducts which are long and much-coiled structures. These should not be confused with the intestine, which is somewhat similar in appearance. Do not remove these oviducts.

Exercise 4. The Digestive Organs

(e) At the very anterior end of the body cavity is the **œsophagus,** which lies dorsal to the three-lobed **liver** and which expands posteriorly into the **stomach.** If possible, push a needle handle from the mouth, through the œsophagus into

the stomach. The stomach passes into the **small intestine,** which consists of several coils, supported by the transparent, sheet-like **mesentery.** Notice the blood vessels in the mesentery. What are the two functions of the mesentery? The small intestine suddenly expands into the **large intestine** or **rectum,** which terminates in the **anus.** The **urinary bladder** is a thin sac connected by a narrow neck to the ventral side of the digestive tract, at the extreme posterior end of the body cavity. The part of the tract between the bladder and the anus is the **cloaca.** It will be exposed in a later dissection.

(f) The liver has already been identified. It secretes a green fluid, the **bile** or **gall,** which is stored in the **gall bladder,** a thin sac on the dorsal surface of the liver. The bile is discharged into the small intestine through the **bile duct** or gall duct which is very small and will not be traced in this dissection. The **pancreas** is an elongated, light-colored organ, lying between the stomach and the first part of the small intestine. It discharges pancreatic juice into the bile duct where the latter runs through the pancreas on its way to the intestine.

(g) Unwind the small intestine, except in the region of the pancreas, by cutting the membranous mesentery; pin it out on the animal's right side, in a position to expose it for drawing. Notice the small, round, dark-red spleen within the mesentery. It is not a digestive organ. Expose the cloaca and bladder as follows: Examine a skeleton to see the form and position of the bone at the base of the hind legs; then with a scalpel, cut straight down through muscle and bone, in the mid-line of the body just back of the bladder and at the base of the legs. **Do not cut too far and mutilate the bladder.** Note carefully the shape and extent of the bladder, and free it from all attachments except where it connects with the cloaca, so that you may determine clearly the form of both cloaca and bladder. Make a drawing (x 2 or 3), showing the entire digestive tract, from mouth to anus, together with its glands, the liver and pancreas.

Exercise 5. Cross Section of Stomach

(h) Remove the digestive organs in the following manner: Cut the stomach across near its anterior end and sever the small intestine just in front of the rectum. Remove the pericardium without injuring the heart which is to be left in place. Remove the digestive tract and the liver, but not the kidneys or other organs.

(i) With a sharp scalpel, cut transverse sections of the stomach, place under water, and study the cut surfaces with a handlens. Identify the following layers: **mucous membrane,** a conspicuous layer lining the cavity; **sub-mucosa,** a thin, clear layer of varying thickness; **muscular layer,** conspicuous and of uniform thickness; **peritoneum,** very thin and to be seen only where it is continued into the mesentery on the outside. Make a sketch (x 5) of the section, showing the several layers.

Exercise 6. Gastric Digestion

(j) The conditions under which digestion occurs in the living animal may be duplicated to such a degree in experiments with non-living material that we can study the digestive process without recourse to the stomach of a living animal. Digestion of food may be carried on in test-tubes if the proper reagents are employed. The process is more rapid at the temperature of the body, but goes on quite actively at room temperatures.

(k) Label four test-tubes as follows: (1) distilled water; (2) distilled water, pepsin; (3) distilled water, hydrochloric acid; (4) distilled water, pepsin, hydrochloric acid. Put in each tube the proper amounts of the materials called for, as directed by the instructor. Put in each tube a small piece of some protein such as white of egg or fibrin. During the laboratory period observe the tubes from time to time and note any changes which indicate that digestion is taking place. At the close of the period, set the tubes aside and again make observations after twenty-four or forty-eight hours. Record your observations and the conclusions drawn from them in the form of a table as explained by the instructor.

Exercise 7. Salivary Digestion

(l) Test the action of saliva on starch as follows: Fill four test-tubes half full with starch paste. Test the tubes Nos. 1 and 2 for the presence of (1) starch and (2) sugar. The instructor will explain to the class how to make these tests. Add to the starch in tubes Nos. 3 and 4 some saliva from your mouth, shake up the tubes and let stand for five minutes then repeat the tests for starch and sugar as above. What are the results? What has been the effect of the saliva upon the starch? Record the procedure and results of the experiment in the form of a table.

Exercise 8. The Absorption of Food

(m) Food, while in the cavity of the digestive organs, is separated from the blood and lymph by the mucous membrane, which lines the digestive tract, and by the thin walls of the capillaries. The food must be absorbed through these membranes before it can be distributed by the circulatory system to all parts of the body. The mucous membrane and the walls of the blood and lymph vessels constitute a semi-permeable membrane, that is, one through which some substances will pass but not others. Some foods are in condition to be absorbed when they enter the body; others must be changed by digestion into substances which can pass through the mucous membrane and the walls of the blood vessels and lymphatics. Parchment and similar animal membranes, which are no longer alive, act as semi-permeable membranes, and may be used in the construction of a simple apparatus to demonstrate the diffusion or non-diffusion of the several food substances. A piece of the parchment is tied over the end of a good sized glass tube. Into this tube is put a solution of the food to be tested and the tube is then lowered into a larger vessel containing water. After some time, tests are made of the water in the outer vessel to determine whether any of the substance in the inner tube has passed out through the parchment membrane.

(n) Such an apparatus will be constructed and demonstrated before the class and the tests to determine the presence

of the substances used will be explained. In one experiment sugar is put in the inner tube. Do tests show that it finds its way through the membrane? In another experiment, white of egg dissolved in water is put in the inner tube. Do tests show that any of this protein has passed through the membrane? Describe the apparatus, the materials used, the method of procedure, and the results. Point out the parallel between this experiment and the conditions in the digestive organs and draw conclusions. What facts may be cited against the physical process of dialysis or osmosis as a complete explanation of the absorption of food in the living animal?

Exercise 9. The Female Urino-Genital Organs

(o) The **ovaries** have been recognized and removed. The two **oviducts** are very long and much coiled. The oviducts do not connect with the ovaries, but each, at its anterior end, opens into the body cavity, by a funnel-shaped opening. To see these it will be necessary to lift up the **lungs**, a pair of brownish, finger-shaped organs lying right and left of the œsophagus. Near its posterior end, each oviduct expands into a thin-walled **sac**, which opens into the cloaca on the dorsal side opposite the bladder. The thin, expanded portion of the oviduct is connected to the body wall, to the rectum, and to the other oviduct by the membranous peritoneum, which must be dissected away to show the real size and shape of this portion of the oviduct. The eggs break from the surfaces of the ovaries and are free within the body cavity, until they pass into the funnel-shaped ends of the oviducts. While within the oviducts, each egg is covered with a gelatinous substance, which swells upon contact with the water, and forms the "jelly" after the eggs have reached the outside by way of the cloaca and anus.

(p) The **kidneys** are a pair of dark-colored, elongated organs attached to the dorsal body wall, and are partly concealed by the oviducts. Carefully remove the right oviduct to better expose the kidneys. The **ureter** is a very slender, light-colored duct which arises from the outer edge of either kidney near its posterior end and extends backward to the cloaca. Do

not confuse the ureter with the several large, white nerves on the body wall dorsal to the ureters or with the large, dark-colored blood vessels which enter each kidney close to its ureter. The **fat bodies** are a pair of yellowish structures, with finger-like lobes, near the anterior end of the kidneys. They are a storehouse of reserve food and do not belong to either reproductive or excretory systems. The yellowish streak on the ventral surface of each kidney is the **adrenal body,** a gland which is not related to the excretory function of the kidney. Make a drawing (x 2) of the female urino-genital organs, the cloaca, and the bladder, as seen from the ventral aspect. It may be well to show only one of the oviducts. Indicate in this drawing the size and position of one ovary by a dotted outline. Now take up work at paragraph (s), Exercise 11.

Exercise 10. The Male Urino-Genital Organs.

(q) The **kidneys** are a pair of dark-colored, elongated organs attached to the dorsal body wall. The **testes** or **spermaries,** as they are also called, are a pair of small elongated bodies suspended from the anterior end of the kidneys. The thin, mesentery-like fold of peritoneum which supports each testis is called a **mesorchium.** The **fat bodies** are a pair of yellowish structures with finger-like lobes, near the anterior end of the kidneys. They are not part of the reproductive or excretory systems, but a storehouse of reserve food.

(r) Running along the outer margin of each kidney is a slender, coiled structure, the **rudimentary oviduct,** corresponding to the oviduct of the female, but not functional in the male. These rudimentary oviducts connect with the dorsal side of the cloaca opposite to the place of attachment of the bladder in the same manner as the functional oviducts of the female. The **ureter** is a slender, light-colored duct which arises from the outer edge of either kidney near its posterior end. Do not confuse the ureters with several large, white nerve cords on the body wall dorsal to the ureters or with a large, dark-colored blood vessel which enters either kidney close to its ureter. The yellowish streak on the ventral surface of each kidney is the **adrenal body,** a gland which is not related

to the excretory function of the kidney. The testes discharge their **seminal fluid** through the **vasa efferentia,** several very slender ducts which can perhaps be seen, along with blood vessels, running through the mesorchium to the kidney. These connect, inside the kidney, with the ureter, through which the seminal fluid reaches the cloaca. Make a drawing (x 2) of the male urino-genital organs, the cloaca and the bladder, as seen from the ventral aspect.

Exercise 11. The Blood Vessels of the Kidneys

(s) Three sets of blood vessels connect with the kidneys: (1) Blood leaves the kidneys through the **post caval vein,** which runs forward to the heart, but which has been cut off, at about the level of the fat bodies, in the removal of the liver. Find its stump and determine its outline between the kidneys. It receives several pairs of **renal veins** from the ventral surface of the kidneys. How does it end posteriorly? (2) Blood enters the kidneys through the **renal portal veins.** One of these arises in each leg and enters the kidney at its lateral margin just anterior to the origin of the ureter. (3) Blood also enters the kidneys from the **dorsal aorta,** through **renal arteries.** The dorsal aorta runs along the mid-line of the body just dorsal to the kidneys, as may be seen by lifting up the outer edge of one kidney. How does the dorsal aorta originate anteriorly and how does it end posteriorly? An **iliac artery** will be observed going to either leg. Make a drawing (x 4) of the kidneys and their blood vessels as seen from the ventral side. Use arrows to show direction of blood flow, and spread the kidneys sufficiently to show, without confusion, the post cava, etc.

Exercise 12. Study of Dorsal Side of Kidneys

(t) Being careful to keep all the parts intact, remove the urino-genitals, their blood vessels, and the cloaca in the following manner: Dissect the anus and cloaca free from the surrounding structures. Cut the renal-portal veins and the iliac arteries. Also cut the dorsal aorta in front of the kid-

neys. With forceps, lift up the cloaca, and working forward, dissect the organs free from the body wall as you go, thus removing the urino-genital organs and that part of the dorsal aorta which is attached to the kidneys by the renal arteries. Place the removed organs under water and study them to determine any points not clear from your former study of the urino-genital system (Exercise 9 or 10). Also make necessary additions or corrections in your drawings of the blood vessels. Make a drawing (x 4) of the dorsal surface of the kidneys, showing especially their relations to the renal-portal veins and to the dorsal aorta. Use arrows to indicate direction of flow of blood.

IV. THE CIRCULATORY SYSTEM

Exercise 13. The Heart and Larger Blood Vessels

(a) Carefully remove any remaining portions of the **pericardium,** the thin membrane surrounding the heart. The heart consists of three chambers: the **ventricle,** a thick-walled, conical portion; and in front of this the **right** and **left auricles,** which are thin-walled and which lie on either side of a single large vessel, the **truncus arteriosus,** which passes diagonally forward from the ventricle.

(b) Blood enters the heart from the body, by way of the **sinus venosus,** which may be seen by lifting up the tip of the ventricle. It is a thin-walled structure formed by the union of three veins: the two **precaval veins** entering laterally and coming from the anterior part of the body; and the **post caval vein,** already recognized. The post caval vein, and perhaps the sinus venosus were removed along with the liver, but the opening from the sinus venosus into the right auricle may still be seen. Blood enters the heart from the lungs by way of the pulmonary veins, which open into the left auricle, but cannot be seen in this dissection. Make a drawing to illustrate these principal veins of the frog, piecing it out from this dissection and from the study of the blood vessels of the kidneys.

(c) Blood leaves the heart through the **truncus arteriosus,** a large vessel which arises from the right side of the ventricle, runs obliquely forward over the ventral surface of the auricles, and then divides into right and left branches. At about one-fourth inch from its origin, each branch of the truncus arteriosus divides into three parts: (1) an anterior branch, the **carotid artery,** which goes to the head; (2) a posterior one, the **pulmo-cutaneous artery,** which leads to the lung, after sending a branch to the skin; (3) a larger, central branch, the **systemic artery,** which runs first in a dorsal, and then in a posterior direction, until it unites with the corresponding vessel from the other side to form the **dorsal aorta.** At the place of union of these two vessels, there is given off a large branch, the **cœliaco-mesenteric artery,** which runs through the mesentery to the digestive organs. A **subclavian artery** arises from either systemic. It will be most easily seen where it passes to the arm, along with a conspicuous, white nerve. When thus identified, it may be traced back to its origin from the systemic. Make a drawing (x 5) of the heart, as seen from the ventral side, together with as many of the arteries as can be made out, including the part of the dorsal aorta removed with the kidneys, and the stumps of the iliac arteries.

Exercise 14. The Capillaries

(d) Examine a demonstration of the circulation of the blood through the capillaries in the web of a frog's foot. Is there any pulse? How can you distinguish arteries, capillaries and veins? What is the relative size of the blood corpuscles and the smallest vessels? Capillaries like these exist throughout the body of the vertebrate animal. Understand their significance in relation to the process of waste, repair, and growth.

V. THE RESPIRATORY ORGANS

Exercise 15. Lungs and Air Passages

(a) Remove the heart, being careful not to injure the **lungs.** Carefully clean off the area between the bases of the

lungs and expose the semi-transparent floor of the **larynx,** the passage which leads to the lungs from the mouth. Probe through the **glottis** with closed forceps and observe the points of the instrument through the floor of the larynx.

(b) Remove the œsophagus, lungs, floor of mouth, and lower jaw, by lifting up the end of the œsophagus where it was cut from the stomach, carefully cutting the attaching structures as you pull, and cutting at the corners of the mouth as necessary. With scissors, separate the removed piece into right and left halves by a cut exactly along the mid-line and passing through the glottis. Observe the size and shape of the larynx as seen from the inside, and find the openings into the lungs. The **vocal cords** are ribbon-like structures attached to the side walls of the larynx. How do they function? Compare this dissection with "half-frogs" prepared by the instructor. Make an outline (x 3) of the head and anterior part of the body of a half-frog, as viewed from the cut side. Add to this outline the mouth cavity, the beginning of the œsophagus, the larynx, and the lungs, as they appear in such a section. Include also all features of the mouth as identified in Exercise 2.

(c) Watch a living frog under a bell-jar. Time the respiratory movements of nostrils, floor of mouth, and sides of body. How does the method of inhaling and exhaling air differ from that in the human body? Make comparisons: (1) with the drawing of air in and out of a pipette; and (2) with the "pumping up" of a tire.

VI. THE NERVOUS SYSTEM AND SENSE-ORGANS

Exercise 16. The Spinal Nerves

(a) On the dorsal wall of the body cavity, the **spinal nerves** are now exposed as whitish cords running outwards from the sides of the **spinal column.** They arise from the **spinal cord,** within this bony protection, and emerge in pairs between the vertebrae, as may be seen by reference to a skeleton. Surrounding the base of each nerve, where it emerges between the vertebrae, is a light-colored mass, the

periganglionic gland. There are ten pairs of spinal nerves designated by numbers beginning anteriorly: The large nerves, previously seen in the examination of the subclavian artery, are the II pair. Trace one of them as far as you can, laying open the muscles of the arm as necessary. The I nerve is a small one just in front of the II. It sends a branch to the II. The III is a small nerve which comes close to the II, sending off a communicating branch. These unions between the first three nerves comprise what is known as the **brachial plexus.** The IV, V, and VI are very slender nerves and run obliquely outward over the muscles of the back. What region do they seem to supply? The VII, VIII, and IX are larger and run almost directly backwards. They have various communicating branches which form a network, called the **sciatic plexus,** as a continuation of which we have the **sciatic nerve** running to the leg. Follow in one leg as far as you can, noticing the blood vessels which accompany the nerve. The X nerve is small, and may be hard to find. It comes out though an opening in the **urostyle,** the elongated bone which terminates the spinal column, and runs almost directly backward to supply the bladder and cloaca. Why are some of the spinal nerves larger than others? Why are there "switch" connections between the nerves of the brachial and of the lumbar regions? Make a drawing (x 2) of the spinal nerves, representing accurately their courses and relative sizes. It may be well to show, in outline, the form of the central portions of the backbone.

Exercise 17. The Ear

(b) Remove the skin from the ear as follows: Make a "V" shaped incision in the skin with the point just in front of the ear and the sides of the "V" passing above and below it. Hold the skin at the point of the "V" with forceps and strip it backwards, exposing a circular area, still covered with a delicate **tympanic membrane** which is thickened at the centre. Cut through this membrane, avoiding the thickened centre, and thus lay open the cavity of the **middle ear,** which

connects with the mouth by the **Eustachian tube.** What is the function of the Eustachian tube? Have you a similar structure? Demonstrate its relation to ear-drum in the human body by holding the nose, closing the mouth, and exhaling. Notice the **columella** of the ear, a slender rod of bone, extending upward and inward from the thickened centre of the membrane to the skull. The function of this is to carry sound waves across the middle ear from the tympanic membrane to the nerve endings in the inner ear. The inner ear is embedded in the bone of the skull and is hard to dissect.

Exercise 18. The Eye

(c) Remove the skin from the dorsal side of the head. Remove the eyeball, noticing the **muscles** by which it is attached to its **socket.** What is their function in the frog's and in your own eye? The **optic nerve** may be recognized entering the back of the eyeball among the muscles. The tough whitish covering on the back of the eyeball is the **sclera,** and is continuous with the transparent **cornea** in front. Notice the **iris** perforated by the **pupil.** What is the use of the iris? With scissors, open the eyeball by a cut from front to back. Study under water. The **lens** is just back of the iris and attached to it. It separates the cavity of the eyeball into two parts: an outer cavity filled with the **aqueous humor**; and an inner cavity filled with the **vitreous humor.** The back of the eyeball is made up of three layers: the **sclera** on the outside; the dark colored **choroid**; the grayish **retina.** The retina is the sensitive part of the eye where the optic nerve terminates. The other parts of the eye are devices to protect the retina and to transmit the light rays to it in the form of a sharp image. Make a large drawing of the eyeball as now opened. After making this drawing, remove the lens, lay it on the printing of your book, and observe the result.

Exercise 19. The Central Nervous System

(d) Cut off the fore limbs close to the body, and the hind limbs by cutting across the trunk at the "waist" line. Tie the four limbs together and put away for a subsequent

study of the muscles. The following dissection will be more readily made if the head and trunk are held in one hand and the flesh pared or cut away with the scalpel or scissors. Remove the skin and muscles from the back, exposing the vertebrae and the skull. Remove also the other eye. With scalpel, shave away the thin bone of the roof of the skull between the eye sockets and expose the **brain.** It will be found covered with a very thin, dark colored, membrane, upon removal of which, the whitish brain will be seen. Notice the large blood vessels running over the surface of the brain and continued posteriorly over the spinal cord. Continue the dissection, working backward until the entire brain and spinal cord have been exposed. What is the importance of the large blood supply to the central nervous system? What is the result if the the blood supply of the human brain is cut off or largely diminished?

(e) There are five main divisions of the brain of the frog as follows, being at the anterior end: (1) the **cerebrum** or **cerebral hemispheres,** a pair of elongated, nearly cylindrical structures, from the anterior ends of which the **olfactory lobes** are indistinctly separated: (2) the **diencephalon,** a short, narrow, depressed area; (3) the **optic lobes,** a pair of prominent rounded bodies; (4) the **cerebellum,** a narrow transverse ridge just back of the optic lobes; (5) the **medulla oblongata,** which gradually tapers into the **spinal cord.** On the dorsal surface of the medulla is a brownish, triangular mass, the **choroid plexus;** or if this has been removed, a triangular depression, the **fourth ventricle,** is exposed.

(f) From the brain there arise ten pairs of nerves, the **cranial nerves,** comparable to the spinal nerves which arise from the spinal cord. In the frog most of these are too small to be seen in this form of dissection, but certain ones may be recognized: The first pair, the **olfactory nerves,** can be exposed by paring away the bone immediately in front of the olfactory lobes. They are short nerves running to the nasal cavities. If not destroyed in the dissection of the eyes, the second or **optic nerves** may be seen by pressing the brain to one side. They arise from the ventral side of the brain. The

fifth or **trigeminal nerves,** which arise from the anterior end of the medulla at the side and run to the face, may be recognized; also the eighth or **auditory nerves,** which arise at about the same place as the fifth and run to the ear.

(g) Push aside the spinal cord and find the **roots** of the spinal nerves, studied in Exercise 16. Carefully remove the brain and spinal cord from the animal and study under water, examining it from its dorsal, ventral, and lateral aspects. The two enlarged places on the cord are the **brachial** and **lumbar enlargements.** To what groups of spinal nerves do they correspond? Draw the brain and spinal cord (x 3) as seen from the dorsal side, including the stumps of such cranial and spinal nerves as have been clearly seen.

(h) Cut away the dorsal surface of the brain and expose the cavities or **ventricles** of the brain. These are all connected with each other and with the central canal which runs the length of the spinal cord. They are found in the brains of all vertebrates and contain a fluid, the **cerebro-spinal fluid.**

Exercise 20. Experiments on the Functions of Different Parts of the Central Nervous System

(i) Watch the demonstrations with frogs which have had successively destroyed: (1) the cerebral hemispheres and diencephalon; (2) the entire brain; and (3) the entire brain and the spinal cord. Write out your **observations** on the behavior of the frog when thus deprived of portions of its nervous system; and your **conclusions** regarding the functions of the several parts of the central nervous system. What seems to be the importance of "intelligent" acts in the daily existence of the frog?

Exercise 21. Muscle and Nerve

(j) Watch the demonstrations of **muscular contraction** in the removed **gastrocnemius** muscle and its nerve, and write out your conclusions regarding (1) the nature of the control over the muscle which is exercised by the nervous system, and (2) the kind of stimuli which affect muscles and nerves. Observe also the action of a heart that has been removed from

the body of a frog or turtle, and write out a statement of the meaning of "death" in the light of these facts.

VII. THE SKELETON AND MUSCLES

Exercise 22. The Skeleton

(a) Study mounted skeletons. Identify the major parts and learn the names of the chief bones by reference to the list given below. Compare the skeleton of the frog with that of man. How do the several parts correspond and how are they modified to suit the life each animal leads? Make a sketch of the skeleton from the dorsal side, showing as many of the bones as possible. The bones of the skeleton may be classified as follows:

I. Axial Skeleton

The **skull** includes: the **cranium** or brain case and the bones of the **face** and **jaws.**

The **vertebral column** is composed of nine **vertebrae** and an elongated **urostyle.** The first vertebra is the **atlas** and has no transverse processes, as have the others. The ninth is the **sacrum,** to which the **hip girdle** is attached.

II. Appendicular Skeleton

The **shoulder girdle** includes: the **sternum** and **episternum,** which together constitute the breast bone; the **clavicle** or collar bone; the **coracoid,** a large bone just back of the clavicle; and the **scapula** (bone) and **suprascapula** (cartilage), which together constitute the shoulder blade.

The **hip girdle,** which is also called the pelvic girdle or the innominate bone, is represented in the frog by a "V" shaped bone formed by the fusion of three, paired bones the **ilium, ischium** and **pubis** of the two sides of the body. The hip girdle is attached to the sacrum.

The Bones of Arm	The Bones of the Leg
Humerus (upper arm)	Femur (thigh)
Radio-ulna (forearm)	Tibio-fibula (shin)
Carpus (wrist), six small bones	Tarsus (ankle), two long bones and several small ones
Metacarpus (hand)	Metatarsus (foot)
Phalanges (fingers)	Phalanges (toes)

Exercise 23. The Muscles

(b) Remove the skin from an uninjured hind leg. The flesh is now seen to be made up of a considerable number of **muscles,** which may be readily separated from each other by using fingers and forceps. These muscles fall mostly into two classes: the **flexors,** which bend a joint, and the **extensors,** which straighten it. Each muscle has a thickened middle portion, the **belly,** and tapers toward the ends, to which are attached the **tendons** by which it connects with the bones.

(c) Study the **gastrocnemius,** the large muscle in the calf of the leg. To what bones is it attached at either end? The large tendon at its lower end is the **tendon of Achilles.** Compare with the same tendon in man and other animals. By moving the joints, determine the relation of this muscle to movements of knee and ankle. Does it serve as flexor or as extensor to these joints? Look on the front of the leg for muscles which "oppose" the action of the gastrocnemius in the ankle region. Observe the two sets of muscles in the thigh. To what bones are they attached at either end? What muscles are brought into play when the frog suddenly straightens the leg, as in jumping or swimming? Remove the muscles of the thigh, noticing their attachments and their mechanical possibilities. Make a diagrammatic drawing (x 2), showing the relation of the gastrocnemius muscle to the bones of the leg; indicate in the diagram or in writing the motions produced by its contraction.

Exercise 24. Joints

(d) Remove the muscles from the **hip-joint.** The joint is not at once exposed, as it is covered by a thin, tough membrane, the **capsular ligament.** This ligament attaches the femur to the hip-bone and also encloses a cavity in the joint, the **synovial cavity,** containing a small amount of fluid, the **synovial fluid,** which serves to lubricate the joint. Cut through this ligament and expose the **head** of the femur. Notice how it fits into its **socket.** In what directions can such a joint move? Study the knee-joint in like manner. How does it differ from the hip-joint. This is a hinge-joint; the hip-joint is a ball-and-socket joint.

VIII. THE USE OF MICROSCOPE

Exercise 25. The Parts of the Microscope

(a) Before attempting to use the compound microscope, one must know the parts of the instrument and their uses. The preliminary work will, therefore, be directed toward practice rather than detailed study of the objects examined. As the microscope is an expensive instrument and one of delicate construction, it must be handled with care, and should always be kept clean.

(b) The object to be studied is placed on a glass **slide,** which is laid upon the **stage** of the microscope. The slide is then moved until the object is over the hole in the center of the stage. Light from a window is reflected **through the object** by means of the movable **mirror.** The observer looks at the object through the **tube** which has **lenses** in each end. The upper lens or **eyepiece** slips into the end of the tube near the eye. The **objectives** are lenses attached to the lower end of the tube. There are two objectives, **low power** and **high power,** one of which magnifies about five times as much as the other. The objectives are attached to a rotating **nosepiece,** by means of which it is easy to change from one to another. Learn to distinguish the "high" and "low" objectives in your instrument, also the high and low eyepieces if your

equipment includes more than one. The instrument may be **focused** by means of two different adjustments: the **coarse adjustment** and the **fine adjustment.** Try these and see how they work. The size of the opening in the stage may be changed by means of an **iris diaphragm,** so that the amount of light may be varied to suit the needs of the object. Some microscopes also have a set of lenses under the stage, forming the **condenser,** a device for giving a stronger illumination.

(c) The surfaces of the lenses must be kept clean, as a very small amount of dirt is sufficient to very greatly decrease the clearness of the image. Even finger prints upon the lenses often interfere with the distinct view of the object examined. Use only the **lens paper** for cleaning the lenses, and if it seems necessary to unscrew the parts of eyepieces or objectives call for the instructor.

Exercise 26. The Use of the Microscope

(d) Clean a slide and coverglass. Place on the slide a small piece of paper cut from a printed page having small letters and smooth paper. Wet with a drop of water by means of a medicine dropper or pippette, and place the coverglass upon the piece of paper. There should be sufficient water to fill the space between the slide and cover, and bubbles of air should not be imprisoned under the cover. It is well to lower the cover with forceps, one edge first, in order to drive out the air.

(e) Have the low power objective in position and a half inch or less above the stage. Place the preparation on the stage and illuminate it properly by moving the mirror into the best position. This can be readily done by looking down the tube after removing the eyepiece. When the light is thus "found", insert the eyepiece and look into the tube with the left eye while the right eye is kept open. The circular area which is now seen is the **field** of the microscope. While thus looking down the tube, **focus** on one of the letters by raising the tube with the coarse adjustment and then securing a more delicate focus by use of the fine adjustment. Adjust the iris

diaphragm, by opening or closing, so that the best results are obtained. Notice that the image is **inverted. Move the slide about on the stage while looking down the tube until you learn how to move it in any direction you wish and are able to place any desired part of it in the centre of the field.** Make an outline of the letter the size it appears to you.

(f) The drawings made by different students will vary greatly in size because they imagine the letter to be at different distances from the eye. To secure uniformity in this respect, imagine that the image is at the level of the table, the place where the drawing is to be made. Lay a pencil on the table close to the base of the microscope on the right side and measure off on the pencil a distance equal to the entire diameter of the circular field. Measure the letter in a similar manner and compare with the size in your figure. Is the letter larger or smaller than you thought it was? It is desirable to use this method of measuring sizes in subsequent work with the microscope in order that you may attain uniformity in the scale of drawings.

(g) To study the preparation with high power proceed as follows: Pick out the part of the preparation you wish to examine; then by moving the slide, place it exactly in the middle of the field of the low power and focus on it sharply. Now, without moving the microscope, rotate the nosepiece until the high objective comes into place. The object will be more or less in focus according to the adjustment of the particular instrument. Bring the object into focus under the high objective by using the fine adjustment only. Use care in this operation, because lens and coverglass come close together and they must not be allowed to touch. When the object is in focus it will be necessary to open the iris a little to get the best results. Make the change from low to high power repeatedly, until you can do it easily and quickly. This is a troublesome operation for a beginner. If it is mastered at the outset, the subsequent work is much easier.

(h) Notice the appearance of the ink of the printed letter and the fibres of the paper. How can you tell whether one

fibre is above or below another? How much of the letter is included in the field of the microscope? Draw circles on the sketch of the letter to show the part included by the field of the high objective: (1) with the low eyepiece; (2) with the high eyepiece. Practice moving the letter about, bringing successive parts to the center of the field. How far is it necessary to turn the fine adjustment to throw the letter completely out of focus? Which way do you turn the fine adjustment to move the tube up or down?

Exercise 27. The Measurement of Objects Studied with the Microscope

(i) Take a small bit of paper cut from a sheet ruled into millimeters. Mount in water and cover as with the letters in (d). By using the pencil and your finger as before, lay off on a sheet of paper the length of a single millimeter as seen: (1) with the low objective and low eyepiece; and (2) with the low objective and high eyepiece. Make several measurements, until you secure a reasonable degree of uniformity. Compare your own results with those of your neighbors. Take an entire sheet of the millimeter paper and lay off two squares: (1) representing the size of a millimeter as magnified by the low objective and the low eyepiece; and (2) as magnified by the low objective and high eyepiece. After completing the two squares, determine the number of diameters the millimeter is magnified in each case; and record on the proper square together with the combination of lenses.

(j) Examine the bit of millimeter paper under the high objective and low eyepiece. The actual field is now less than one millimeter in diameter. To measure the fraction of a millimeter which this field represents: Move the slide so that one edge of the field coincides with one of the ruled lines; notice a fibre of the paper which is conspicuous and which coincides with the opposite side of the field; move the slide until this fibre reaches the side which was first occupied by the line; and repeat this operation until the edge of the field reaches the next ruled line. When you have established the size of

the field in terms of the millimeter, add to the sheet of millimeter paper a line representing the magnified length of one millimeter as seen under this combination of lenses. Determination of the magnification with the high objective and the high eyepiece will not be attempted. It is impossible with the paper. It is, however, easily made by the use of a stage-micrometer, an instrument which consists of a glass slide having one or more millimeters and their subdivisions ruled thereon. In the method you have employed, the bit of millimeter paper has been used as a stage-micrometer.

(k) Preserve the sheet of millimeter paper as a record. Now or later a measuring stick for microscopic objects may be made by laying off, upon a strip of cardboard, the millimeters as magnified by each combination of lenses. Objects seen at any subsequent time may be projected upon a sheet of paper close to the stand, and the cardboard measuring stick used in place of the pencil. With a little practice, any object which occupies a substantial part of the field, may thus be measured with a surprising degree of accuracy.

IX. HISTOLOGY OF THE FROG

ISOLATED TISSUES

Exercise 28. Squamous Epithelium from the Frog

(a) The outermost layer of the frog's skin may be seen "sloughing off" when living frogs are confined in a small aquarium of clear water or it may be obtained from the liquid in a jar of preserved specimens. The outer layer of your own skin is continually sloughing off in a similar manner, except that it falls away in microscopic particles. Examine a bit of this material from the frog, placing it on a slide in a drop of water. Spread out the filmy mass without tearing; add a cover; and study first, with low, and then with high power. Notice the **cells,** each with a distinct **nucleus** surrounded by **cytoplasm.** There may be more than one layer of these cells. These cells in the preparation are only the outer

part of the skin of the frog. The entire skin is much thicker than this and will be studied in a future exercise. What do you conclude regarding the relative thickness of these cells as compared with their length and breadth? Measure the size of one of these cells by using the scale made upon the mm. paper. Make an accurate drawing of a few of the cells, picking out a typical place and drawing the cells exactly as they appear. Size of drawing at least one inch for diameter of each cell. Record the actual size of the cells as above measured.

Exercise 29. Squamous Epithelium Cells from the Human Mouth

(b) After cleaning a blunt edge such as the handle of scalpel or forceps, scrape the inside of the cheek, and mount the material thus obtained in a drop of saliva. Before covering, scrape away as many of the bubbles as possible. In this way cells will be obtained which are very similar to those studied above, but are separated from each other. Find cells seen from different views, so that you may convince yourself that they are really flat. Draw isolated cells, as seen from edge and from flat surface. Size at least one inch in diameter.

Exercise 30. Columnar Epithelium from the Frog's Intestine

(c) Examine, in a drop of the alcohol, a bit of the frog's intestine which has been cut from a fresh specimen and soaked for twelve or fifteen hours in 30 per cent alcohol, a treatment which causes the cells to fall apart. Take one of these short pieces of the intestine; cut it open; and scrape off the mucous membrane, discarding the muscular layer. With needles, separate the material thus obtained into a powdery substance; add a cover; and examine with low and high powers. Elongated cells will be found arranged in groups and singly. Determine the exact shapes of typical cells, and how the cells are fitted together. What is the exact appearance of the nucleus? Make a drawing of a typical cell and of several cells grouped together. Size two or more inches for length of the cell.

Exercise 31. Ciliated Columnar Epithelium from the Frog's Mouth

(d) Examine in salt solution a bit of the mucous membrane from the roof of the mouth of a recently killed frog. Scrape off the softer material on the mouth side of the piece, discarding the tough membranous material; and add a cover after further teasing of the sticky mass which remains. With low power, look for movement appearing in different parts of the mass and resembling the flickering of a flame. Or the vibration of the small detached particles which are being driven about by the cilia may first attract attention. Examine such a place with the high power and study the action of the cilia in mass and upon single detached cells. The cells are rather short "columns," tending to be pointed at their inner ends and blunt at their outer, ciliated ends, but contracting when the living cells are torn apart and appearing as globular or bluntly cone-shaped bodies. The cilia cannot be seen individually; but they may be recognized as a flickering zone, bordering the free ends of the cells. Observe the action of the cilia and its effect upon any small particles which may be in the preparation. Look for dying cells whose cilia are no longer in motion or have entirely disappeared. In such cells the nucleus may be distinguished. It is of relatively large size. Draw one or more of these cells. Size two inches in length.

(e) Observe, in a demonstration, the action of the cilia upon small objects placed upon the roof of the mouth; and compare with their action upon microscopic particles. What is the function of these cilia?

Exercise 32. Striated Muscle

(f) Take a small bit of muscle from a freshly killed frog and place it at once in a watchglass half full of normal salt solution. Do not at any time give the material the least chance to dry. With needles fray out the piece, separating but not injuring the individual fibres which may be seen with the eye. (1) Stain a small mass of fibres as follows: Put a drop of **methyl violet** on one end of a slide and a drop of water

on the other; with forceps, put several fibres into the stain and leave them there for about half a minute; then transfer them to the water to wash off the surplus stain; mount them in a clean drop of water on another slide. If the preparation is successful, the nuclei will be stained a deeper purple than the rest of the fibre. (2) Mount a few fibres in salt solution without staining. Use these two slides in the following studies. For nuclei, the stained material should be used; for other features, the unstained fibres will be more satisfactory.

(g) Each fibre is a single cell, but one with a large number of nuclei. What about the length of such a cell? Each fibre is composed of a large number of very minute **fibrillae,** which cause it to show rather indistinct longitudinal markings. There is a very much more distinct **transverse striation,** the feature from which this kind of muscle derives its name. The fibre is enclosed in a very delicate membrane, the **sarcolemma,** which cannot be recognized except at places where the fibre is somewhat injured or at the end of the fibre. Draw a portion of a fibre to show all these features. Size at least one-half inch in diameter.

Exercise 33. Non-striated or Smooth Muscle

(h) Examine a piece of the digestive tract of the frog which has been properly macerated to separate the fibres. Tease thoroughly and mount. The cells are elongated, tapered towards the ends, and each has a prominent nucleus near the middle. How do these fibres compare in size and shape with striated muscle fibres? Draw a typical cell. Size at least two inches in length.

Exercise 34. Cartilage

(i) Examine in salt solution sections of cartilage that have just been cut, with a sharp knife, from the head of the femur of a recently killed frog. After locating with the low power the thinnest part of the section and distinguishing the cartilage proper from the regions in which bony material is deposited, study with high power. Cartilage is a tissue having its cells

widely separated and the intervening mass or **matrix** of a transparent, rubber-like material. The cartilage cells lie in holes called **lacunae** within the matrix. In some lacunae there may be seen two cells; or two lacunae may be seen close together with flattened adjacent sides, indicating that cell division has recently taken place. Can you also find cells in fours? In eights? Draw, showing cells and matrix. Size of cells about one-half inch in diameter.

Exercise 35. Connective Tissue

(j) Examine a small piece of connective tissue, spreading it out flat on a slide in a drop of water, adding a cover after fraying out the edge a little with needles. This tissue is most readily obtained from between the muscles of the leg in a preserved frog or from the inside of a cat's skin which has been preserved in formaldehyde. Connective tissue resembles cartilage in having widely separated cells with a large amount of intercellular material, but differs from it in that this material is in the form of very fine fibres. There are two kinds of these fibres: **white fibres,** very fine ones which run in wavy bundles; and **elastic fibres,** which are thicker, occur singly, and are straight. In some preparations one and in some the other type of fibre predominates. In many preparations it will be hard to distinguish the two kinds. The cells of connective tissue are not easy to demonstrate but they may be seen by staining. To stain this material, remove the cover and stain, as in the case of the striated muscle fibres, with methyl violet. Make a drawing to illustrate connective tissue.

Exercise 36. Bone

(k) Pieces of dried bone, ground to thin sections, will be used. In these, only the inorganic substance of the bone remains; but the extent and distribution of the **bone cells** is shown by the cavities which the cells once occupied. These cavities, or **lacunae** appear black, because in the grinding of the section they become filled with dirt and air. Examination with high power will show: the lacunae as elongated, black

areas which were formerly occupied by cells; and radiating from them, fine black lines, the **canaliculi,** which in life were occupied by delicate processes of the bone cells. Compare the structure here observed with that seen in cartilage. In some bones, the cells are grouped about canals in which run blood vessels. These are termed **Haversian canals,** each of which with its surrounding cells is an **Haversian system.** Stained sections of decalcified bone show the cells and their nuclei. Examine, if available. Draw, showing the points observed. Size of lacunae or cells about one-half inch in length.

Exercise 37. Blood of Frog

(l) Place a drop of the frog's blood on a slide. Cover at once, before it has time to begin to dry, as drying changes the appearance of the cells. Blood is a tissue composed of two kinds of cells foating in a fluid, the **plasma.** What is the color of the **red cells** or **corpuscles** when seen singly? Determine their shape by observing them from different angles. The nucleus is readily seen. The **colorless cells,** or **leucocytes,** are much less numerous and very transparent; they are much smaller than the red cells. Some of them will be seen to be irregular in shape; and you may possibly see that some of them change shape while you watch them; their nuclei cannot be recognized. Make a model, with a piece of oil clay, showing the shape of each kind of cell. Make drawings of red cells in different positions, to show their shape. Make drawings of the colorless cells. Size of red cells about two inches in the longer diameter, white cells in proportion.

(m) Stain a fresh preparation with **methyl violet** by placing a small drop of the stain at the edge of the coverglass and allowing it to run under. Study especially the colorless cells in which the nucleus is now visible. Add this to your drawing.

(n) Watch the circulation of blood in the web of frog's foot, looking for the two kinds of corpuscles, and noting the relative size of blood cells and capillaries.

Exercise 38. Human Blood

(o) Secure the blood as follows: Sterilize a needle by passing it through a flame. Wrap a finger tightly with a clean handkerchief or towel, beginning at the base, so that the tip becomes distended with blood. Prick with a needle just back of the base of the nail till you get a good sized drop. Touch a slide to it and cover at once. Compare with frog's blood in regard to size and shape of **red cells.** Do you find a nucleus? Observe that the cells may be arranged in rows like piles of coins. These are called **rouleaux.** Do the **colorless cells** seem to differ in number or appearance from those of the frog? Make a clay model of a red cell. Make drawings showing the red cells, both singly and in groups. Size of cells about one inch in diameter.

(p) Stain human blood as follows: Place a drop of methyl violet on your finger and prick through the stain allowing the blood to be stained as it flows from the wound. Mount at once. Make necessary drawings or notes.

TISSUES COMBINED IN ORGANS

Exercise 39. The Skin of the Frog

(q) The skin will be studied in a permanently stained and mounted section cut at right angles to the surface. Double staining has been employed, so that the nuclear substance is stained a different color from the cytoplasmic substance. The skin is made up of two layers: the **epidermis** on the ouside and under it the **dermis** or **corium.** The epidermis is a **stratified squamous epithelium.** Its superficial cells are much flattened, and the deeper ones become progressively less so. On the surface is the layer of very flat cells which you studied from a surface view in Exercise 28. The dermis is composed of connective tissue with numerous blood vessels and two layers of cells containing **pigment.** Notice the large **glands** opening to the exterior by **pores** or **ducts.** To which of the two layers of the skin do the cells lining the glands belong? What is the function of these glands? Have you anything comparable

in your own skin? What is the function of these glands in the frog? Make a good sized drawing of a narrow vertical strip through the skin, showing in detail the structure of the layers and including a gland which is so cut as to show the duct. Label fully.

Exercise 40. The Wall of the Digestive Tract

(r) The intestine of the mud-puppy Necturus is better for this study than the intestine of the frog. The structures observed are found in all vertebrates. On the slide are very thin sections cut across the intestine, and showing the structures as they actually lie in place. Compare with the rough sections of the stomach which were previously studied (Exercise 5). As with the section of the skin, double staining has been employed. Identify the gross features of the sections with the handlens, before studying with the low and high powers of the compound microscope. There are five layers in the wall of the intestine: On the outside is the **peritoneum,** composed of a single layer of squamous epithelium, here seen from the edge. Beneath this is the **longitudinal muscle layer,** of smooth muscle cells, cut across in the section. This is followed by the **circular muscle layer,** similar to the longitudinal layer, except that the fibres run around the intestine and are, therefore, cut lengthwise. Next to this is the **submucosa,** which is composed of connective tissue and contains many large blood vessels. What is their function? What is the structure of their walls? Do any of them contain blood cells? The inner layer, bounding the cavity, is the **mucosa** or **mucous membrane.** It is composed of a single layer of columnar epithelium. In this layer are seen many **goblet cells,** each with its drop of mucus. Can you find cases where the mucus is being secreted into the intestine? Between the submucosa and the epithelium of the mucosa is a thin layer of smooth muscle cells, the **muscularis mucosae.** This is considered a part of the mucous membrane. Understand thoroughly the structure of each of these layers and be able to explain the part each plays in the functioning of the digestive tract. A part of the **mesentery** is usu-

ally seen attached to the section. Which layer of the intestine forms the outer layers of the mesentery? What tissue forms the middle layer of the mesentery? Are there blood vessels in the mesentery? Make two drawings to show the above points: (1) A simple outline about three inches across, showing the general form of the section and its layers, but no details within the layers. (2) A full page drawing of a narrow strip across the wall of the intestine, showing the details of structure as they appear under high power. Label thoroughly, giving the names of the layers and the tissues of which each is composed and any other features of interest.

UNICELLULAR ANIMALS AND PLANTS

I. THE AMŒBA

PHYLUM, PROTOZOA. CLASS, RHIZOPODA

Exercise 1. Occurrence and Collection

(a) Many species of the genus Amœba and its close relatives occur in fresh-water, others in moist soil, and some as parasites in the digestive tracts of larger animals. The fresh-water amœbas are often found in abundance, but the cultures are difficult to control and the amœba is, therefore, not so easily secured at specified times as are many other unicellular forms. The ooze from the bottom of a pond or stream, the floating scum, the slime adhering to the stems and leaves of the larger water plants, and similar material not infrequently contains great numbers of amœbas. If you have opportunity, it is interesting to see the actual collection in the field and how the cultures are handled. In any case, examine several laboratory cultures, which contain amœbas and other protozoa, noting the general appearance of the material, its color, odor, and the like.

Exercise 2. General Structure and Activities

(b) Fresh material from a culture known to contain amœba will be placed on slides and distributed. Use a very small

aperture in the diaphragm and look with the low power for small, transparent objects containing minute granules. After finding such an object examine under high power. If it is an amœba, the characteristic flowing movements will be recognized. If an amœba is not readily discovered, look at one under a neighboring microscope or ask the instructor to show you a specimen. The amœbas are not difficult to find in a good culture, although other forms are often mistaken for them. When a specimen is located, slowly open and close the iris diaphragm and watch result. Why it is necessary to use a small aperture in searching for amœbas and similar transparent organisms? Watch the changes in shape. Does the animal accomplish a definite locomotion by this means? The processes which flow out from the cell are called **pseudopodia,** i. e., "false feet". Make four drawings showing the outlines assumed by a single individual at intervals of one minute. These drawings should omit all internal structure and show outlines only, the direction of the flow being indicated by arrows.

(c) Begin a large drawing, by making an outline three or four inches across; and, as you proceed, add the items noted in the following sections. Examine the substance composing the unicellular body. There is an outer region of clear **ectoplasm** and an inner mass of granular **endoplasm.** Some of the larger masses in the endoplasm are surrounded by a clear drop of fluid. Sometimes the contents of such watery drops or **vacuoles** can be recognized as green plant cells, similar to the ones found in the water outside. These spaces are **food vacuoles** or "temporary stomachs" in which food is being digested. Examine the other granules of the endoplasm, determining their size and shape. Determine the nature of the boundary between ectoplasm and endoplasm by watching the formation of pseudopodia.

(d) A single large vacuole may be identified as the **contractile vacuole,** if it is seen to contract quickly and then to expand slowly. This contains no particles, only fluid which is drawn from the endoplasm and then forced to the outside. It is not easily recognized in the smaller specimens.

(e) The **nucleus** is seen with difficulty in the smaller individuals, but is usually recognized in larger ones as an oval mass, finely granular, transparent, and about the size of the expanded contractile vacuole. What is the exact shape of the nucleus as seen in a large amœba? If the nucleus is not seen clearly in the living animal, examine a demonstration of a stained and permanently mounted specimen.

(f) If individual amœbas are carefully watched, it is sometimes possible to observe the manner in which food is ingested and fecal matter egested.

(g) Add to the general drawing such of the above details as you have actually seen, labeling all parts.

(h) Examine amœbas in a drop of water on a slide, without a coverglass and under the highest magnification obtainable with low objective. What can you make of the vertical dimension? Correct any errors in previous figures and make a clay model to show the superficial features.

Exercise 3. Special Structures and Activities

(i) If large specimens are abundant, study the contractile vacuole as it appears and disappears, and represent by a series of figures; study also the currents within the endoplasm and the changes in external contour by which single pseudopodia are formed and withdrawn. Drawings are desirable.

II. THE EUGLENA

PHYLUM, PROTOZOA. CLASS, MASTIGOPHORA

Exercise 1. Occurrence and Collection

(a) Species of the genus Euglena are common in fresh water, being sometimes present in such numbers as to cause the green or reddish color in the bottom ooze or surface scum of ponds and sluggish streams. If you do not have opportunity to see the material collected, examine the cultures containing euglenas, noting their general appearance and the parts of the vessels in which they are most abundant.

Exercise 2. Active Phase

(b) Fresh material from the cultures will be distributed on slides. Look with the low power for elongated, green bodies which may be at rest or moving about. Put one under the high power and observe the form and movements. Can you distinguish an anterior and a posterior end? A slight notch in the profile of one end marks the opening of the **gullet,** within which is attached the **flagellum,** a long thread-like process difficult to see when in motion. Understand from textbook or lectures how this is used in locomotion. Find individuals which are expanded and others which are contracted; or better, observe how a single individual expands and contracts. Make a clay model to show these external features.

(c) Begin figures three or four inches long, showing the outlines of: (1) a contracted; and (2) an expanded euglena. As you proceed, add details to the latter drawing, labelling fully.

(d) Continue, examining favorable specimens. Do you find a **nucleus?** What other structures can you find inside the cell or on its outer surface? There is a spot of red pigment at the anterior end, the so-called **eye-spot.** The lighter spot in this portion of the cell is caused by a group of vacuoles which function as do the **contractile vacuoles** of other forms.

(e) Examine demonstration specimens, showing the nucleus.

(f) If the flagellum has not been observed, prepare another slide and stain with iodine or methyl violet. Look for the flagellum at the anterior end and add this to the diagram.

Exercise 3. Encysted Phase

(g) Encysted euglenas are often found in cultures which have been standing for some days in the laboratory. Examine such materials, and note **pigment spot, nucleus,** and **cyst.** Do any of the individuals shows signs of **cell division?** Understand from lectures or textbook the relation between the two phases of the life-cycle. Draw one or more individuals in this encysted condition.

III. THE PARAMŒCIUM

PHYLUM, PROTOZOA. CLASS, INFUSORIA

Exercise 1. Occurrence and Collection

(a) Paramœcium is one of the unicellular forms most easily obtained. The species **P. caudatum** is commonly studied. In nature, the animals are most abundant where the water is foul and ill-smelling, as in streams having a large amount of sewage and other organic material in process of decomposition. Examine the laboratory cultures and note how they differ from cultures of amœba and euglena. The animals may be seen with the eye as moving, white specks. Do they tend to collect at certain places in the culture jars?

Exercise 2. General Structure and Activities

(b) Fresh material will be distributed on slides, without coverglasses. Do not add a cover at first; but examine with the low power, noting the rapid movements and general behavior. Watch a single individual as it moves about. What determines the direction of its locomotion? Does the animal act as though it profited by experience? Determine the exact shape. How does the anterior end differ from the posterior? What is the shape and position of the **buccal groove,** which extends backward from the anterior end on one side of the body? At what point does this groove pass within the body as a blind tunnel, the gullet? Is the animal bilaterally symmetrical? Disregarding for the present the granular contents of the body, make a clay model showing only the external features. With the aid of the model and specimens examined without a coverglass, make a drawing **showing the external features only.**

(c) Put a very small number of absorbent cotton fibres upon a drop of water containing paramœcia and add a coverglass. The animals will be caught in pens, formed by the meshes of the cotton, and kept within a limited space. Find

an animal which is thus enclosed, but not in any way crushed, and examine with low and high powers. As it becomes quieter, make out: the **cilia,** which cover the surface of the body and cause locomotion; the **contractile vacuoles,** two clear vesicles which appear and disappear; **food vacuoles,** scattered through the body and having variously appearing contents; and the granular substance of which the cell is composed. Add the cilia to your previous drawing; and then begin a large figure, three or four inches long, showing the internal structure. Put in all points thus far observed and add others as you proceed.

(d) Make a fresh mount, without cotton, and stain with methyl green or aceto-carmine. Look for a **nucleus.** Does the nucleus react differently from the **cytoplasm** when the stain is applied? What does the staining indicate regarding the chemical or physical composition of nucleus and cytoplasm? The conspicuous nuclear structure, seen by this means or in the living paramœcium, is the larger or **macro-nucleus.**

(e) Examine a demonstration showing the smaller or **micro-nucleus.** In life this is imbedded in a depression at one side of the macro-nucleus and cannot be recognized with certainty by the crude staining method applied to the living animals as in (d).

Exercise 3. The Trichocysts

(f) Look in this same preparation for specimens in which many stiff processes, much longer than the cilia, have been extruded from the cell as a result of contact with the stain. If not found, try another slide, adding a drop of stain to the water on the slide before putting on the cover. These are the **trichocysts.** They are used for defensive purposes. How? Draw a small portion of the body margin, on a large scale, showing trichocysts and cilia; or these may be shown on a part of the margin in the figure of the internal structure.

Exercise 4. The Food Vacuoles

(g) Take a very small drop of water, containing many paramœcia, and add an equal amount of water containing In-

dia ink or finely powdered carmine. Watch for a time and see whether any ink gets into the cell. Where and how? Study also the action of the locomotor cilia, as they drive the particles about. Examine any ink which has entered the body and observe how the food vacuoles originate. How is the ink contained within the cell? Can you see the actual "gulping down" of the particles? In this fashion food enters the paramœcium. A drawing is desirable.

Exercise 5. The Contractile Vacuoles

(h) Mount some specimens in a very small drop of water, holding them in place, without crushing, by the weight of coverglass. Study the formation and collapse of the contractile vacuole and time the contractions. Write an accurate description, accompanied by three drawings showing stages in the process.

Exercise 6. Finer Structure of the Protoplasm

(i) Put a small drop of water containing paramœcia upon a slide and cover with a slip. Draw off the water with filter paper until the animals are brought under pressure, but not crushed. Examine with the high power looking along the margin of the cell for rod-like bodies, the **trichocysts** before discharge. The firm line outside these is the **cuticle.** The trichocysts lie in the **ectoplasm.** Draw off the water until the animal is crushed and the semi-fluid **endoplasm** flows out. Examine this mass with highest power and see what you can make of it. To what extent does the visible structure explain the activities of this unicellular organism?

Exercise 7. Reproduction

(j) Individuals are often seen in process of reproduction. This occurs by the **binary fission** of the cell. If such a specimen is found, it may be kept under observation by moving the slide about; and after some minutes will be seen to separate into two independent cells, each of which subsequently becomes a perfect individual. Examine demonstrations,

showing the division of the nuclei. By this process of cell division reproduction is accomplished. Draw, if observed.

Exercise 8. Conjugation

(k) Paramœcia may be found in process of **conjugation.** They are easily recognized as pairs which swim about adhering together by their buccal grooves. Study and draw such pairs if found.

(l) Examine demonstrations showing the nuclei during conjugation. Understand from lectures or textbook what happens to the nuclei and what is the probable significance of conjugation. A drawing is desirable.

IV. GREGARINA AND MONOCYSTIS

PHYLUM, PROTOZOA. CLASS, SPOROZOA

Exercise 1. Occurrence and Preparation of Gregarina

(a) Many protozoa live as parasites in the bodies of other animals. Notable among these, are the gregarines, which occur in the digestive tracts of arthropods. Material for this study may be obtained from the larvae or adults of the meal beetle, **Tenebrio.** Take a living larva of the beetle on a glass slide and snip off, with scissors, the last segment of the body. With forceps, tear off the head, endeavoring to pull out the entire digestive tract along with the head. The digestive tract may then be placed on the slide and the other parts discarded. Without adding a coverslip, examine the transparent tract with low power. The gregarines, if present in numbers, will be seen within the tract as dark colored bodies, two or three times longer than broad. Look for a digestive tract containing a considerable number of the gregarines. When found, such a tract may be chopped or teased to pieces in a very small drop of salt solution before adding the cover. If your neighbors are less fortunate, a single digestive tract may be used for the making of several preparations. As the infection with

any parasite is largely a matter of chance, a number of trials may be necessary before satisfactory material is obtained. Avoid getting much of the creamy fat-body of the insect mixed with the bits of the digestive tract; and use the salt solution sparingly, as the gregarines live longest when in the fluid of the gut cavity.

Exercise 2. Active Stage of Gregarina

(b) The gregarine has sharp outlines because of its firm cell membrane. The cell is divided into two parts, a smaller **protomerite** and a larger **deutomerite.** Where is the nucleus? Is there **ectoplasm** and **endoplasm**? What is the nature of the protoplasmic structure? Do the organisms move, and how? Do you find more than one type? Individuals are often found attached end to end. This is not cell division, nor conjugation, but merely a habit of the animals as they plow through the thickish fluid in which they live. Draw a good sized figure or figures to show the above. Compare the gregarine with the paramœcium. How is the structure and activity of each related to its environment?

Exercise 3. Encysted Stages of Monocystis

(c) Encysted stages are not readily obtained in the case of **Gregarina**; but may be found in individuals of the genus **Monocystis,** a sporozoan which lives in the seminal vesicles of the earthworm. Here the encysted stages are abundant and the active stages present in lesser numbers.

(d) Take a bit of the seminal vesicle from a fresh earthworm and tease out in salt solution on a slide. Add a cover and look for spherical bodies, containing two hemispherical cells or many smaller spindle-shaped objects. These are the encysted stages of this gregarine. Look for active stages. The latter are large, spindle-shaped, naked cells. Have they a nucleus? Protomerite? Deutomerite? By reference to textbook or to instructor's explanation in laboratory, identify as many stages as your slide affords and draw the same arranged in order.

V. YEAST AND BACTERIA

ASCOMYCETES AND SCHIZOMYCETES

Exercise 1. Occurrence and Preparation

(a) Yeasts and Bacteria are unicellular plants which are very widely distributed in both their active and their encysted stages. Their occurrence in the decomposing organic material which constitutes their food, in the dust of the atmosphere, and under a wide range of conditions will be appreciated after the completion of the study which is here outlined. For this work, yeast is obtained by adding a small bit of yeast cake to Pasteur's solution, a nutrient fluid containing the chemical compounds essential for the growth of "yeast plants". After a few hours, the resting stages of the yeast cake have become active and have produced, by cell division, large numbers of cells which continue to multiply as long as sufficient food material remains. Brewers' yeast is a variety which is preferable to that of the ordinary yeast cake, because the cells are of greater size and the cell divisions more conspicuous. Bacteria will be found in the cultures along with the yeast cells, but may be obtained in greater variety from cultures prepared by adding pond water to such organic substances as hay, bread, beans, gelatin, beef broth, and the dead bodies of animals.

Exercise 2. Structure of the Yeast Plant

(b) Get a drop from a yeast culture, add a coverglass, and examine with low and high powers. Countless oval cells, the "yeast plants", will be seen. Is there a definite cell-wall? What structures are found in the protoplasm? The **nuclei** of the yeast cells cannot be seen without special staining. Do you find the cells in process of division? Compare with the binary fission of paramœcium. This form of cell division is called **budding**. How does yeast grow and reproduce itself? Draw a single cell, showing the detailed structure; also a

colony or group of cells in outline only. Size about two inches in diameter.

Exercise 3. Experiments in the Growth of Yeast

(c) Clean three test-tubes: Fill the first two-thirds full with distilled water; the second and third two-thirds full with Pasteur's solution. Label the tubes. To each tube add a little yeast taken from a culture. Plug each tube with cotton. Boil the second tube after plugging, and do not remove the plug after the boiling. Note the appearance of each tube and construct a table to record same as observed on this and subsequent days. Set all three tubes aside for examination at next laboratory period.

(d) After 24 or 48 hours, examine the tubes, without unplugging, to determine the changes visible with eye alone or with handlens. Record in table. Unplug and take a sample of the solution from each tube. Place on separate slides, cover, and study with microscope. Do you find yeast plants in each tube? Record in table. What conclusions do you draw regarding the conditions under which yeast grows? This experiment is now completed and the tubes should be washed and returned.

Exercise 4. Bacteria in the Active Stage

(e) Examine under low and high powers a drop of fluid from an "infusion" which has been standing several hours and become turbid. Find the cause of the turbidity. Draw off most of the water from under the cover and study the objects with a good light and highest power. Is there any movement? Is there any active translation from place to place? These minute bodies are mostly living bacteria. Study several slides if necessary, making out their form and proportions. Can you see any nucleus or other internal structure? Draw on a large scale to show, in outline only, the shapes of as many distinct types as you discover. By the methods explained on p. 27, estimate the size of some of the larger bacteria.

(f) **Examine** various cultures and infusions, observing the conditions under which bacteria in the active state may be very abundant. Record in your notes the kind and the relative abundance of the bacteria, and the general conditions in each culture examined.

Exercise 5. Resting Phases of Bacteria and Yeast

(g) Examine demonstrations showing **spores** in yeast and bacteria. Draw. What is the importance of such stages in the life-cycle?

Exercise 6. An Experiment with the Dust of the Air

(h) In this experiment, either a watchglass containing a gelatinous culture medium or a cooked potato will be used. In the latter case, potatoes that have been in a steam sterilizer for one hour will be given out. Cut the potato in halves with a knife heated in a flame. Lay the halves directly upon the surface of a sterilized glass plate, with cut surface upward. Be sure that nothing except the heated knife touches the cut surface. Leave cut surface exposed to air of laboratory for one hour. At end of this time cover with a sterilized finger bowl. Paste labels with your name on glass plate and finger bowl, as the potato will remain on table for several days. Each time you come into the laboratory examine the surface for any changes visible to the eye. When growths appear scrape off a little of the material with a sterilized knife, dilute with sterile water and examine under the microscope. Why does this experiment fail to prove conclusively that the bacterial and other growths, which have appeared upon the potato, have come from the dust of the air? Do you think bacteria, yeasts and moulds are more widely distributed in their active or in their resting condition? Write out in your notes the conclusions you can now draw, regarding the floating matter of the air and the conditions under which the spores of bacteria, yeasts, and moulds will germinate; also any applications to the protozoa. When the experiment is completed wash and return the glassware.

SIMPLE MULTICELLULAR ANIMALS

I. THE HYDRA

PHYLUM, CŒLENTERATA. CLASS, HYDROZOA

Exercise 1. Occurrence and Collection

(a) Two species of Hydra are commonly found: **Hydra viridis,** which is green; and **Hydra vulgaris,** which is usually gray, though at times brownish. The hydras live in ponds of clear water, and are most readily collected when attached to small objects or to the stems and leaves of pond plants. In the fall, they are often found in great numbers attached to the dead leaves which have sunk to the bottom of a pond. When such material is brought into the laboratory and allowed to stand in aquarium jars, the hydras can be seen attached to the vegetation or to the walls of the jars. Such cultures should be examined in order that the animals may be seen under approximately natural conditions.

Exercise 2. External Features

(b) Study a hydra in a watchglass with sufficient water to allow the animal to expand properly. Use handlens and lowest power of compound microscope. The animal is attached by one end, the base. The free end of the cylindrical body terminates in a conical elevation, the **hypostome,** about which are several **tentacles.** How many? In the end of the hypostome is the **mouth,** which is likely to be tightly closed unless the animal is feeding. Notice the knob-like swellings on the tentacles. These are clusters of **stinging capsules** which will be studied later. Observe the movements and changes of shape of the animal and make a drawing to show its external features when it is well extended. Make also an outline drawing of a contracted hydra.

Exercise 3. General Internal Structure

(c) Notice that the body is hollow, containing a space, the **digestive** or **enteric cavity** into which the mouth opens.

but the three barbs appear pressed together and resembling a miniature spearhead. Look carefully on the edge of the tentacle for thorn-like processes, the "trigger-hairs" or **cnidocils.** These are projections from the cnidoblasts. Draw, showing the above.

(g) Run a drop of saffranin under the cover and remove the filter paper so that the animal will be crushed. This causes the discharge of many more nematocysts and it also stains some of them. Look for nematocysts of much smaller size than those studied above, and having a thicker thread without barbs. These are deeply stained and can be seen in both their discharged and undischarged condition. If necessary, tap on the cover to cause further breaking up of the animal. Other types of nematocysts may be seen by using the highest magnification. Make drawings of these smaller types of nematocysts.

Exercise 6. The Cells after Maceration

(h) Macerate a hydra with Bela Haller's fluid as follows: Place the animal on a slide with very little water, and add a drop of Bela Haller's fluid. After one-half minute, quickly remove the fluid, with filter paper, and add at once a drop of methyl violet. After about two minutes remove the stain and add a drop of water. Break up the hydra by teasing with needles, and put on a cover. If necessary, tap gently on the cover with a needle to further separate the cells. In this way the cells may be separated from each other while the individual cells are left intact.

(i) The following types of cells will be recognized in favorable preparations: (1) **Endoderm cells,** the largest ones present, usually elongated and always with a conspicuous vacuole or vacuoles in the cytoplasm; (2) **Gland cells,** elongated, slender cells with deeply stained, granular cytoplasm; (3) **Ectoderm cells,** somewhat smaller than the endoderm cells and rectangular in shape; (4) **Interstitial cells,** very small, rounded cells, with relatively large nuclei; (5) **Stinging cells** or **cnidoblasts,** perhaps already recognized and studied. It may

The wall surrounding the cavity is composed of two layers of cells, the outer of which, the **ectoderm,** is thinner and more transparent than the inner layer or **endoderm.** In the green hydra, the endoderm is bright green and the ectoderm colorless. Can you distinguish these layers of cells? Are there extensions of the enteric cavity in the tentacles?

(d) Study prepared cross sections of the hydra. The two layers of cells will be recognized and between them a thin layer, the **supporting lamella,** which is not composed of cells. Make a diagrammatic drawing of this section, and also a diagrammatic figure to show the structure of the animal as it would appear in a longitudinal section passing from end to end and including a tentacle.

Exercise 4. The Ingestion of Food

(e) For this exercise it is necessary to use hydras that have been made "hungry" by keeping them without food for at least twenty-four hours. Use as food **small bits** of the flesh of a frog, crawfish, water snail, or raw beef. To induce the animal to take the food, use a clean needle and push a small bit of the food against the tentacles or close to the mouth. Watch the animal throughout the process, using hand-lens and lowest power of compound microscope. Make a series of sketches to show how the ingestion is accomplished.

Exercise 5. The Cnidoblast Cells and their Nematocysts

(f) Mount a hydra on a slide, under a coverglass supported at one edge by a very small piece of filter paper. Examine with low power of compound microscope. Clear, ovoid bodies, the nematocysts, are seen in the tentacles, and less abundantly in other parts of the animal. Each nematocyst is contained within a cell known as a **cnidoblast.** Tap on the cover and induce some of the nematocysts to "discharge". In the discharged condition, a long, slender **thread** with **barbs** near its base is seen projecting from a bulb-like capsule. In the undischarged condition, this thread is coiled up inside the capsule. This coil is not readily seen in the large nematocysts,

be possible to find some of these last in which the capsule has not been torn from the cell, in which case the cytoplasm and nucleus can be recognized. Draw typical examples of as many of these cells as you can identify.

(j) From these laboratory studies, what conclusions can you draw regarding cell specialization and the relative development of tissues and organs in the protozoa, the hydra, and the frog?

Exercise 7. Reproduction by Germ Cells

(k) Sexual organs appear periodically on the hydra, although they are absent during the greater part of the year. The mature **ovary** contains a single **egg,** surrounded by a protective **shell.** The **testes** are hemispherical organs, each containing many **spermatozoa.** Make drawings.

Exercise 8. Reproduction by Budding

(l) Make a sketch or sketches of a **budding hydra,** from living or stained preparations, as material is available.

Exercise 9. Regeneration

(m) Clean two watchglasses, filling one of them two-thirds full of water, from a jar in which hydras have been living, and using the other as a cover. Take several hydras and cut each transversely into two or more pieces. Examine the pieces with low power of compound microscope. Set aside and examine at subsequent laboratory periods until the regeneration is complete. The phenomenon of regeneration is widespread, but is most highly developed among those groups of animals which reproduce extensively by means of vegetative processes such as budding and fission. Compare with the well known powers of regeneration and vegetative reproduction in plants.

II. THE HYDROIDS AND HYDRO-MEDUSAE

Exercise 1. Occurrence and General Structure of Colony

(a) Hydroids are marine cœlenterates closely resembling hydra in their general structure. They live attached to rocks, seaweed, submerged woodwork, and the like. They differ from hydra in that the individuals, instead of living singly, live together in **colonies** comparable to the colony which would be formed if a hydra budded many times and all the buds remained united to the parent body.

(b) For this study, preserved specimens of the hydroid **Obelia geniculata** will be used. Examine, with a handlens, a portion of a colony in a watchglass of water; and also museum specimens of entire colonies of **O. geniculata** and other hydroids. In some of the colonies, the individuals are large enough to be recognized without a lens. Examine the obelia with lowest power of compound microscope. There are two kinds of individuals which are permanent members of the colony: (1) **Feeding individuals** or **hydranths**; (2) **Reproductive individuals** or **blastostyles.** There is thus a "division of labor", among the individuals of the colony. The feeding individuals, which are the more numerous, are those with tentacles. Immature feeding individuals with rounded ends will also be seen. The reproductive individuals are the club-shaped structures found near the base of the stem. Notice how the upright stems are fastened at the base and how root-like, horizontal stems extend over the surface on which the colony is attached. Draw (x 2), showing these general features of a portion of a colony.

Exercise 2. Feeding Individuals or Hydranths

(c) Select a fully matured hydranth that is properly expanded and identify the parts as found in the hydra: **body, hypostome, tentacles, enteric cavity, ectoderm,** and **endoderm.** Notice that the body of the hydranth is continued downward as a slender stem, the **cœnosarc,** which is continuous with the

common cœnosarc of the colony. The ectoderm, the endoderm, and the cavity of the hydranth are thus continuous throughout the branched stem, and its root-like extensions.

(d) The entire colony is protected and supported by a thin, transparent covering, the **perisarc,** which is not present in hydra. The perisarc of the stem is continued upward to form a cup-shaped **hydrotheca** about each hydranth. Notice the shelf-like expansion of the hydrotheca on which the hydranth rests; the ringed form of the perisarc just below the hydrotheca; and certain places where the perisarc is greatly thickened. Draw a single hydranth, on a large scale, including its connection with the upright stem.

Exercise 3. Reproductive Individuals—Blastostyles and their Medusæ.

(e) The blastostyles above noted are without mouths or tentacles. How are they nourished? The covering of a blastostyle is the **gonotheca.** How does it differ in shape from the hydrotheca? Attached to the blastostyle are numerous rounded bodies, the **medusa buds.** A medusa bud when fully matured becomes a **medusa** or jelly fish which is detached from the blastostyle and escapes into the water through an opening at the end of the gonotheca. The medusa may be considered **a third type of individual** in the colony, which unlike the other two types (hydranths and blastostyles) is not a permanent part of the colony. What process in the hydra is comparable with the detachment of the medusa from its parent hydroid colony? Draw a single blastostyle on a large scale, showing its connection with the main stem and also the medusa buds in several stages.

(f) Examine demonstrations of the medusae of obelia, in the stages just after detachment, when they are swimming freely in the water. In the later stages of the medusae, reproductive organs appear, as may be seen in a large hydroid medusa such as the species **Gonionemus murbachii.** Draw the medusa of obelia.

Exercise 4. The Life-cycle

(g) The obelia colonies reproduce only by the vegetative method—in this case by **budding.** They never have sexual organs as do hydras. The medusæ, on the other hand, have sexual organs and reproduce by means of eggs and spermatozoa. The offspring produced by the medusæ are not medusæ, like their parents; but are hydranths which, by extensive growth and buddings, develop into a colony like the one just studied. The life-history of obelia thus exhibits an **alternation of generations,** since the attached hydroid colony alternates with the free-swimming, medusa. How could the life-cycle of an extensively budding hydra be modified to give stages comparable with those in the life-cycle of obelia? Construct a table or a full page diagram comparing the life-cycles of the obelia and the hydra.

III. A LARGE HYDRO-MEDUSA

Exercise 5. Occurrence and Habits

(a) The medusæ of obelia are very small and not easily studied. The larger hydro-medusa **Gonionemus murbachii,** which is almost identical in structure and essentially like the obelia in its life-history, is a more favorable specimen. The preserved medusa may be studied, under water in a watch-glass, using handlens and lowest power of microscope. Handle the specimens carefully so as not to injure the fragile structures. The appropriateness of the popular name "jellyfish" will now be appreciated. At the close of the period, return the specimen if it remains uninjured.

(b) This medusa lives in the shallow water of protected inlets. It originated by detachment from a simple hydroid colony which is attached on the bottom. Compare with the life-cycle of obelia. In life the gonionemus is often seen "fishing" for its food by swimming up to the surface, turning mouth side uppermost and slowly settling to the bottom with tentacles widely extended. If a small fish or similar animal comes in contact with the tentacles it is quickly paralyzed

by nematocysts and drawn to the mouth. Individual medusæ sometimes show the enteron above the hypostome greatly distended with food. Compare with hydra. What part would be played by such a free living stage in the life-cycle of an attached animal?

Exercise 6. Structure

(c) The medusa is umbrella-shaped with the **hypostome** in the position of a short, thick handle. The margin bears many **tentacles** which are well supplied with stinging cells. The **velum** is a circular shelf projecting inward from the margin of the medusa, so that it partly closes the **sub-umbrellar cavity.** Notice the four, much convoluted sexual organs, **ovaries** or **testes** according to the sex. The hypostome is perforated by the **mouth** which communicates with a **stomach** from which extend four **radial canals,** one above each sexual organ. The radial canals communicate with the **circumferential canal,** at the margin of the disc. At the base of each tentacle is a colored, **eye spot.** Between these organs, clear vesicles, the **lithocysts** or organs of equilibrium, may be seen with the compound microscope. The animal is covered on the outside with ectoderm, and the cavities entered through the mouth are lined with endoderm as in hydra. Between ectoderm and endoderm is a thick mass of "jelly", the **mesoglœa,** which corsponds to a much thickened supporting lamella such as is found in hydra. Make a drawing (x 4) of the animal as seen from the oral or concave side. Construct a diagrammatic vertical section (x 4) in the plane of two opposite radial canals.

PARASITISM AND OTHER FORMS OF ASSOCIATION AMONG ANIMALS

I. A PLANARIAN WORM

PHYLUM, PLATODA. CLASS, TURBELLARIA

Exercise 1. Observation of the Living Animal

(a) Planarian worms are common in fresh-water, where they are most easily discovered on the under sides of leaves, stones and small objects upon the bottom. The species **Planaria maculata** is excellent material for the work here outlined.

(b) Examine in a watchglass of water. How does the animal move? What changes in shape may the body undergo in "righting"? In meeting obstacles? In response to other stimuli? What is the shape and distinctive feature of each end? Are there sense organs? Can you find the **mouth**; and, in sexually mature animals, the **genital aperture** on the ventral side? Transparent specimens will show the dendritic branches of the **digestive system,** the plan of which should be understood from chart or textbook figures. Place a small specimen upon a slide under a coverslip and look for **cilia.** Can you see the justification for applying the name turbellaria to these forms? Study also the coloration under microscope and handlens. Draw the animal from a dorsal view (x 10), showing the above features.

(c) Specimens will sometimes feed if crushed snails or bits of meat are placed in the dish. The muscular **pharynx** may then be seen. In this connection, their actions may be watched for evidence of a **chemical sense.** Interesting observations may also be made upon **regeneration** and upon their behavior with respect to light. Carry out such observations and experiments if you have time.

(d) These worms are studied with a view to emphasizing their **free-living** condition. In nature, they move about actively, often capturing living prey; and reproduce either

vegetatively, by **fission,** or by means of **germ-cells.** They are **hermaphroditic.** The eggs are laid in small, stalked capsules which are attached to the under sides of the stones and other objects upon which the animals are living. **From each of these capsules or eggshells a number of young emerge, as miniature adults, able at once to take up the life of the parent upon the bottom.** Their life-cycle is thus in marked contrast with the life-cycles of the parasitic representatives of this phylum.

II. A FLUKE-WORM

PHYLUM, PLATODA. CLASS, TREMATODA

Exercise 2. Structure and Parasitism of the Adult Worm

(e) To the **trematoda** belong the external and internal parasites known as the **fluke-worms.** For this study, any species of the genera which resemble **Distomum** may be used. Specimens of the genus **Pneumonœces,** from the lungs of the frog, or of the genus **Clinostomum,** which is found in a somewhat immature condition encysted in the cœlomic region of the frog, are excellent material.

(f) Examine living or preserved specimens and locate the **mouth** and **suckers.** How does the shape and behavior, if specimens are observed alive, compare with the same in the planarian? Locate the **digestive tract** and compare with the planarian. The **reproductive organs** are complex and varied in appearance. If studied, special directions will be given. The animals are **hermaphroditic** and produce fertilized eggs which accumulate in a terminal portion of the female organs, the **uterus,** developing later when they are laid. The life-cycle is greatly complicated by the parasitism as described in lectures or textbook. Both in the structure and conditions of the life-cycle, contrasts should be drawn between the trematode and the planarian. Make a figure showing the features above noted.

III. A TAPE-WORM

PHYLUM, PLATODA. CLASS, CESTODA

Exercise 3. The Adult Worm

(g) The **cestoda** or tape-worms are parasitic forms, even more highly modified in relation to their parasitic habits than are the fluke-worms. The adults occur as parasites within the digestive tract of another animal; the larval stages mostly within the tissues of a **secondary host** upon which the **primary host** is likely to feed. Species of the genus **Tænia** occur in many common mammals and are excellent for the study of the external features. They may be examined alive in water or after preservation in alcohol or formalin.

(h) Examine an adult cestode in a pan of water. The smaller end has a head or **scolex**; the posterior end ripe joints or **proglottids.** What structures, adapted for holding fast to the host, are found upon the scolex? Count the proglottids, compare with numbers in neighboring specimens, and record. Can you see indications of the developing reproductive organs, and of the **genital apertures**? How and where do the proglottids seem to originate? **Each proglottid contains a complete hermaphroditic reproductive system.** The chain of proglottids may be regarded as a reduplication, many times over, of the reproductive machinery. If living worms are available, test the firmness of their attachment to the mucous membrane of the host. Why is it important that the adult cestode maintain its place within its host? Compare the external features with those of other tape-worms shown as museum specimens. **Moniezia expansa,** from the sheep, and **Crossobothrium laciniatum,** from the sand-shark, are good for this purpose. Draw a good sized figure of the adult, indicating the parts as above and with the proglottids accurately shown. To avoid repetition, the figure may show representative regions connected by dotted outlines.

Exercise 4. The Six-hooked Embryo

(i) The "ripe" proglottids at the posterior end often show the outlines of the distended **uterus,** a cavity in which the development is begun. Here, the egg develops as far as the **six-hooked embryo,** a stage which may be obtained from either living proglottids or formalin material. Cut the proglottid into bits in a watchglass and examine some of the material under high power of the microscope. Embryos surrounded by a tough **shell** and other membranes will be found. Can you find the six **hooks**? Is there any remnant of the **yolk** material? If alive, crush by pressing on the cover and watch movements. Do they seem effective as boring movements? Can you make an estimate of the number of six-hooked embryos produced by a single cestode? Draw showing these features. Size about two inches in diameter.

Exercise 5. The Bladder-worm

(j) The ripe proglottids, with their six-hooked embryos, break off and pass out with the feces of the host. The six-hooked embryos are discharged by the rupture or disintegration of the proglottid and find their way to the secondary host by the chances of nature, entering with the food or drink. After its membranes are digested in the stomach of this host, the six-hooked embryo bores out into the tissue and develops to a stage known as the bladder-worm. Examine living or preserved material and make out the **scolex** and **neck** and their peculiar position in the **bladder.** Can you see anything adaptive in this development of the scolex within the bladder? Understand what happens when the bladder-worm is eaten by the primary host. Draw the bladder-worm, making a good sized figure.

Exercise 6. The Internal Structure

(k) If the internal structure of the proglottid is to be studied, excellent results may be obtained from the motile proglottids of **Crossobothrium laciniatum** stained with alum

cochineal. Further explanations will be given in the laboratory.

(l) Other important features in the internal structure of the cestode, which may be noted in chart or textbook figures or in a further laboratory study are: the **brain** and **nerve cords**; **excretory system**; **absence of a gut**; the granules of **$CaCO_3$**; and the **cuticular membrane** which covers the body. Are any of these features to be correlated with the parasitism?

Exercise 7. Representative Life-cycles

(m) Tape-worms have two hosts. In the following cases of known life-cycles, which would be the primary and which the secondary host? Man—pig; dog—rabbit; mouse—cat; house-fly—hen; fish—bird; louse—dog; man—fish. Consider where and how each of the three stages would occur in each case. Tabulate your conclusions.

IV. OTHER FORMS OF ASSOCIATION AMONG ANIMALS

Exercise 8. Comparison between Parasitism and Commensatism, etc.

(n) The platoda offer an excellent example of closely related animals in the free living and in the parasitic state. From what you know regarding these and any other parasitic forms, write out a statement of the kind of modifications, in structure and life-history, likely to occur in animals which have assumed parasitic habits. Consider: cases of **communal parasitism** in insects, as explained elsewhere; the striking cases of association occurring among marine animals, such as crabs, worms, and mollusca; and the whole in relation to lecture, textbook and field work bearing upon the inter-relations among different species and the struggle for life.

THE EARTHWORM

PHYLUM, ANNUATA. CLASS, OLIGOCHÆTA

I. BEHAVIOR AND EXTERNAL FEATURES

Exercise 1. General Activities

(a) The following directions apply to any of the species of **Lumbricus.** Place a vigorous, active worm upon wet filter paper in a dissecting pan and carefully observe the mode of locomotion. How does it elongate and contract? Can you see stiff spines projecting from the sides? Can they be drawn in? Is there a rhythm in these changes? Draw the worm through the fingers and feel the spines, or **setæ.** How many are there on one ring? Place the worm on its back. Does it right itself? Will it crawl backwards? Compare the anterior and posterior ends, the dorsal and ventral, the right and left sides. Which are alike? Touch various parts of the worm to see which seem the most sensitive. Note the movements of the soft lobe, the **prostomium,** above the mouth. On the mid-dorsal line look for the blood vessel which shows through the skin. Does it pulsate? Which way does the blood move? Hold up the worm to the light and see the dark central axis which is the digestive tract with its contents. On the ventral side may be seen light colored swollen areas, the **skin glands.** Those on some segments may be associated with a smooth swollen band passing around the animal, the **clitellum.** On the 15th segment, swellings mark out transverse slits on each side, the openings of the **vasa deferentia** or ducts for the discharge of sperm.

Exercise 2. External structure

(b) For this study a preserved worm will be used. Observe the structural features already observed in the living animal. Count the entire number of segments or **somites** and record, comparing with counts on neighboring specimens.

What segments are occupied by the clitellum? By means of the handlens examined the setæ in defferent regions. Locate the anus. Look on the 14th somite, near the ventral setae. for the minute openings of the oviducts. Make a figure (x 2), showing the anterior end as far back as a point just behind the clitellum, from a ventral view. Number segments and locate all structures observed. Make a similar figure of the posterior end showing 10 segments from a ventral view.

II. GENERAL INTERNAL STRUCTURE

Exercise 3. The Digestive Tract and Cœlome

(a) Fasten down, **dorsal side up,** in a dissecting pan, by pinning through the first somite and again toward the posterior end. Make an incision about one and a half inches long, just back of the clitellum and **on the mid-dorsal line,** but do not cut too deep. Using fine scissors cut toward the head end with great care to cut no deeper than through the body wall and to keep on the dorsal mid-line. Spread out the edges of the cut body wall and pin them apart after breaking the transverse partitions, **septa,** which connect the inner surface of the wall and the outer surface of the gut. **Slant the pins outward to give room for fingers and instruments in working.**

(b) You will now be able to see the brownish **intestine** and, on its upper surface, the large **dorsal blood vessel.** Toward the head, the gut becomes differentiated and is partly hidden by other organs which will be indicated presently. Between the body wall and the digestive tract is a space, the body cavity or **cœlome.** Thin lines pass from the digestive tract to the body wall across the body cavity. With the handlens and a needle, one may see and feel that these are the edges of the septa which divide the body cavity into chambers one behind the other. What is the relative position of septa and external rings?

(c) Continue the cut forward as far as the second segment. Carefully separate the edges of the cut and see the different regions of the digestive tract. Identify the following, begin-

ning anteriorly: **pharynx, œsophagus, crop, gizzard,** and **stomach-intestine.** In the sexually mature animal there are large yellowish-white lobes in certain segments; these are the three pairs of **seminal vesicles.** They more or less hide the œsophagus. The so-called **hearts** are located in segments 7 to 11. These hearts are merely pairs of blood vessels which arise from the dorsal vessel and encircle the œsophagus in their passage to the median **ventral blood vessel.** On top of the digestive tract in segment 3 is a small white body, the **brain.** Spread out the body wall right and left and pin it to the wax, slanting the pins obliquely outward as before. In doing this, break or cut some of the septa with a needle or scissors. How do the anterior septa differ from the others? Have they any different use? In all but a few of the anterior chambers of the body cavity there are paired fluffy masses on each side. These are the **nephridia** or excretory organs. Look with a lens for fine blood vessels on these organs. Turning a part of the intestine to one side, you may be able to see that these fine vessels are connected with the median ventral vessel. Beneath the ventral vessel is a conspicuous band, the **nerve cord.** Cut the specimen open for its entire length and carefully separate the various organs to see them more clearly. Which segments are differentiated? Which ones merely repetitions of similar organs. Make a full page diagram of the region from the prostomium to the beginning of the stomach-intestine to show all the organs thus far made out. The length of the segments may be exaggerated and the organs drawn as if separated by dissection. Number the segments and locate all the organs in their proper segments.

(d) Lift up the œsophagus with forceps, carefully cutting its attachment to the septa. Cut it across near the pharynx and pull it gently back, while cutting off the septa. How are the seminal vesicles placed with reference to the œsophagus? Be careful not to remove the seminal vesicles from the worm while continuing to pull back the digestive tract and to cut the septa. Continue this as far back as the beginning of the stomach-intestine; and so lift up and remove from the worm

the œsophagus, crop, gizzard and part of the intestine in one piece. Examine this removed portion of the tract under water and correct any errors in your previous drawing. Find the **calciferous glands,** three pairs of lateral pouches on the œsophagus in the region hidden by the seminal vesicles.

Exercise 4. The Cœlomic Fluid

(e) Clean a slide and cover and, with the aid of an instructor, draw out a drop of the **cœlomic fluid** from a living worm by means of a capillary pipette. Place immediately upon the slide, adding salt solution if necessary, and examine with high power. Find the cells of the cœlomic fluid. What are their characteristic activities? What organism do they resemble? Have they nuclei? Draw, showing characteristic shapes. Size one to two inches across cell.

Exercise 5. The Excretory Organs

(f) Using fine scissors, remove a part of a septum with a nephridium attached and examine under low and high power. The nephridium is a convoluted tube with interlacing blood vessels. Look for the ciliated funnel, or **nephrostome.** Understand the function and manner of action of the nephridium. With the aid of an instructor, obtain a bit of a living nephridium, or better, one that is complete. Look for the peculiar flickering movement of the **cilia** within the tubule and study this with the high power. Draw the nephridium in whole or in part as observed.

Exercise 6. The Reproductive System

(g) The earthworm is an hermaphroditic animal, that is one in which male and female reproductive organs are found in the same individual. The female system consists of **ovaries, oviducts,** and **seminal receptacles;** the male system of **testes, seminal vesicles,** and **vasa deferentia.**

(h) Wash off the region of the reproductive organs by gentle currents from a pipette, and then examine the posterior face of the septum between segments 12 and 13. Under a handlens, the two **ovaries** may be seen lying one on either side and near the nerve cord. Immediately behind each ovary is

an **oviduct,** seen as a whitish area on the front face of the septum between segments 13 and 14, and in segment 14 as a fine cord which is very short and passes diagonally outward to its place of exit on the ventral body wall. Locate these parts without pulling away the remains of septa and nephridia and then make them more clear by gently pulling or cutting any tissue which renders them obscure. Examine a model and understand how the eggs pass from ovary to oviduct.

(i) Examination of the **seminal vesicles,** which should still be uninjured, will show that the three lobes which extended up on either side of the œsophagus are united by a common median region which lies below the gut and against the ventral body wall. A little picking away of this middle region will disclose four large bodies, rather indistinct in outline, but different in texture from the vesicles and resembling crumpled bits of paper. These are really greatly modified **funnels** which lie at the beginning of the male ducts, or **vasa deferentia.** Looking on the ventral body wall and outside the seminal vesicles, it is possible to find a fine duct running out laterally from the region of each funnel. The two on a side unite and pass straight back to the opening of the vas deferens on segment 15. By means of these funnels and ducts the sperms pass out of the seminal vesicles. The sperm funnels really open within the closed cavity of the seminal vesicles. The sperms originate from the **testes,** bodies somewhat similar to the ovaries and in the same relative position in segments 10 and 11. Although the testes lie close to the openings of the sperm funnels, the sperms upon dropping from the testes do not at once enter the funnels, but pass up into the lobes of the seminal vesicles where they develop to mature spermatozoa, which are then ready to enter the funnels and vasa deferentia and so pass to the outside. The four testes and the four funnels have therefore a relation to the cœlome similar to the ovaries and their oviducts, while the seminal vesicles by enclosing both testes and funnels in a common cavity prevent the spermatozoa from entering the cœlome and furnish a cavity in which the spermatozoa complete their development.

(j) The **seminal receptacles,** which should not be confused with the seminal vesicles, are small whitish bodies attached to the ventral body wall on either side in the region of segments 9, 10 and 11. They open to the outside only, and their function is to retain the spermatozoa obtained, during copulation, from another worm.

(k) Consult a model, or a figure of the entire system, and then construct a large semi-diagrammatic figure showing all these parts. Review their relationships by tracing the course of the ova and spermatozoa from their origin to the external openings of oviducts and vasa deferentia.

(l) Carefully cut out one of the ovaries and transfer to a slide. Add a drop of glycerine, put on a cover and study under the low power. The **ova** will be seen in various stages of development. Where are they most advanced? The largest ones show clearly a **nucleus** and **nucleolus.** Make a drawing two or three inches in length showing the entire ovary.

(m) Understand from lectures and textbook the functioning of the various parts in copulation and egg laying. Examine again the **clitellum,** and the markings which extend forward from this to the openings of the vasa deferentia and the external openings of the oviducts.

Exercise 7. The Nervous System

(n) Lift the posterior end of the pharynx with forceps and cut the muscles that connect it with the body wall. Trace the nerve cord anteriorly and find where it divides into right and left branches which encircle the pharynx and unite in the **brain.** Look for the nerves from the brain and from the collar-like **connectives** around the pharynx. Determine the number and place of exit of the nerves arising from the ventral cord in the region just back of the "collar" and in the body at posterior end. Cut across the nerve cord in mid-body region and remove a bit of it by tearing out with a quick pull of the forceps. This piece may help you to determine the number of nerves per segment. Make a diagram (x 5) of the nervous system from a dorsal or a lateral view. Indicate the relation of the nerves to the segments.

III. THE STRUCTURE AS SHOWN BY SECTIONS

Exercise 8. The Gross Structure

(a) Take a piece of an undissected specimen about an inch in length and using a sharp pair of scissors, cut transverse sections about one segment in thickness. Study under water with the handlens and make out the position of **gut, cœlome, nephridia, septa, nerve cord, blood vessels,** etc. Can you distinguish different layers in body and gut walls? Notice the **typhlosole,** a fold of the dorsal wall of the gut. How may it be of importance in digestion and absorption? Make a drawing about three inches across, which will show the structures appearing in the region midway between septa.

Exercise 9. The Cellular Structure

(b) Study mounted sections of this same region. Examine first with handlens and then with the low power to make out the appearance of the parts. Then study the cellular structure of each part with the high power.

(c) In the body wall there are four cellular layers: (1) The **epidermis,** composed of columnar epithelial cells, some of which are **gland cells** in various stages of activity. Covering the outer surface of these cells, is a continuous membrane, the **cuticle,** of non-cellular nature and produced as a secretion from the epidermis. (2) A layer of **muscle fibers** which encircle the body and are, therefore, cut lengthwise in this section. Are nuclei and blood vessels discernable? (3) **Muscles** running in a longitudinal direction constitute the next and thickest layer of the body wall. They are so arranged as to have a feather-like appearance when the groups of fibers are seen in this section. (4) The innermost layer is the **peritoneum,** the lining of the body cavity. It is a single layer of squamous epithelium, which in favorable sections may show cell outlines and nuclei.

(d) In the intestine there are three chief layers: (1) The innermost, the **mucous membrane,** of very narrow columnar cells. Do they bear cilia? (2) The outermost, the **chlorogogue**

layer, appears as a granular mass which may be resolved into much modified columnar cells of varying length. (3) The middle layer, a narrow band of **muscle fibers,** both longitudinal and circular.

(e) Examine the section as a whole. The typhlosole will again be recognized. What is the condition of the three main layers in this region of the gut? The region of the cœlome is likely to be confusing, because of the irregular manner in which the septa and nephridia are cut. Try to understand these parts and why they appear as they do in your section. Locate the **dorsal, ventral, sub-neural,** and **lateral neural** blood vessels.

(f) Draw on a large scale a narrow strip of the body wall to show the cellular structure of each of the foregoing layers, and on the same page a similar part of the intestine located in the right position relative to the body wall. Add anything made out in the cœlome.

(g) Study the nerve cord in the above section and note: The outer layer, containing **muscle fibers**; the three large, clear areas on the dorsal side, the **giant fibers**; the **nerve fibers** of the main mass; and the **ganglion cells.** Understand from lectures or textbook the nature of the connections between the cells of the nervous system. Make a drawing of the nerve cord as seen in cross section. Size about two by three inches.

Exercise 10. Comparison of the Cell-layers and Tissues in Frog and Earthworm

(h) Compare layer for layer the body and intestinal walls of the earthworm with corresponding parts of the frog. Construct a table showing: the names of the several layers of each; and the kinds of cells of which each layer is composed. What do you regard as the fundamental differences between the body-plan of an invertebrate and that of a vertebrate, as shown by cross sections of frog and earthworm? What important features are common to both?

THE INSECTS

PHYLUM, ARTHROPODA. CLASS, INSECTA OR HEXAPODA

I. THE GRASSHOPPER OR LOCUST

Exercise 1. Observations upon the Living Animals

(a) The grasshoppers and locusts are the most common representatives of the order **Orthoptera,** and any large specimen of several species, which are common locally, may be used. Living individuals should be observed in glass jars, containing grass and covered with a screen. Exactly how are the legs used in walking and jumping? The **spiracles,** or respiratory openings will be seen along the sides of the abdomen. Observe and time the intervals between the respiratory movements. Note the nature and the distribution of color upon the animal. Can you suggest any value which this may have for the animal in nature? Offer bits of green vegetation to the specimens in the jars and see what you can make out regarding their mode of feeding. Touch the "feelers", **antennæ,** of the head with a long piece of glass tubing having a plug of absorbent cotton in the end and observe how sensitive to touch are these organs as compared with other parts of the body. Moisten the absorbent cotton with some strong-smelling fluid and bring it near the antennae without touching them. Can the animal smell with these organs or with any other part of the body? Remove a specimen from the jar and examine the parts at closer range. Look at the **compound eyes,** the antennæ, etc., with the lens. Note the "molasses" which is regurgitated from the mouth. This is a digestive fluid mingled with food. If a good-sized drop can be collected from one or more specimens and placed upon a slide, put a bit of fresh, green vegetation in this and note result before the fluid evaporates. What may be the significance of this habit of regurgitating the contents of the digestive tract? If you have time, devise experiments to determine whether temperature,

or sensations akin to fear in the higher animals influence the rate of the respiratory movements.

Exercise 2. The General External Features

(b) The body of the grasshopper has three main divisions: the **head**; the **thorax,** which bears the legs; the **abdomen,** which is without appendages. Each of these divisions is made made up of a number of more or less well-defined rings called **segments.** The outer covering or **exoskeleton** protects the internal organs and serves as a place of attachment for the muscles. The exoskeleton is composed of a horny substance called **chitin.** Bend the animal and observe that the exoskeleton is not absent but only thinner at the joints, both in the body and in the legs. The entire outer surface of the animal is thus covered by a continuous armor-like skeleton. The skeleton of each segment is composed of two chief parts: the **tergum,** or dorsal part, and the **sternum,** or ventral part. Compare this sort of skeleton with that of the frog or man, which is an internal, or **endoskeleton.**

(c) The body consists of three main regions: the **head**; **thorax**; and **abdomen.** Though the head is made up of several segments, they are so closely united that they cannot be distinguished. On the head are two large **compound eyes.** Examine the surface of one of them with a handlens and see that it is divided into a large number of small areas, each corresponding to one of the small independent visual units of which the large eye is composed. There are also three simple eyes, or **ocelli,** which may be seen with the lens: one of them just in front and near the top of each compound eye; the third one in the median line between the bases of the feelers. The skeleton of the top and front of the head is the **epicranium.** Below this is the **clypeus** from which is suspended the **labrum** or upper lip. The side of the head below the compound eye is the **gena.** The head bears four pairs of appendages which will be studied in greater detail later: (1) the feelers, or **antennæ**; (2) the **mandibles,** or jaws, which are covered by the labrum and partially exposed on either side of the head

just below the genæ; (3) the **maxillæ,** each of which bears a short jointed feeler of palp; (4) the **labium,** or lower lip, which also bears a pair of short palps.

(d) The thorax includes three segments: the **prothorax, mesothorax,** and **metathorax,** each of which bears a pair of legs. The tergum of the prothorax has the form of a hood which extends backward some distance over the mesothorax. Each leg of the grasshopper has the following five divisions: the **coxa,** a short segment by which the leg articulates with the body; the **trochanter,** also a short segment (not distinct in the large jumping leg); the **femur,** a long segment; the **tibia,** a long segment bearing spines; the **tarsus,** which is divided into several parts and which ends in a pair of **hooks** and a little **pad.** Cut into the femur of one of the large jumping legs and see the muscles. The thorax bears two pairs of **wings,** a pair of tough, thick ones and a pair of thin membranous ones. On which segments are they?

(e) The abdomen includes ten segments and is without appendages. Segments 2—7 in the female, and 2—8 in the male, are complete rings and essentially alike. The first segment is interrupted at the sides by a backward extension of the metathorax which bears the third pair of legs. The dorsal portion of the first segment bears the **tympanic membrane,** a sense organ whose function is supposedly auditory; its ventral part is only indistinctly separated from the metathorax. The terga of the ninth and tenth segments are very narrow and only partially separated from each other. Just back of the tenth tergum is the **dorsal plate,** beneath the tip of which is the anus. At the sides of the dorsal plate are the two triangular **podical plates,** and at the base of each of these a small projection, the **cercus.** In other respects, the terminal portion of the abdomen is different in the two sexes. In the **female,** the abdomen terminates in two pairs of stout, pointed structures which form the **ovipositor,** used for digging the holes in the ground in which the eggs are laid. In the **male,** the sternum of the ninth segment is prolonged backward and upward as the **genital plate.**

(f) On each of the first eight abdominal segments is a pair of **spiracles,** the openings of the internal **tracheæ** or **respiratory tubes.** These are seen along the sides of the abdomen on the ridge between the sternum and the tergum. The spiracle on the first segment is just in front of the tympanic membrane. There are two pairs of spiracles on the thorax, located in the thin membrane between the segments.

(g) Make a large drawing of the insect as seen from the side. Spread out the wings above the back in such a position as to show their size and shape; arrange the legs in about the position they would assume when at rest; number the segments of the abdomen and label all structures.

(h) Make a drawing of the head as seen from directly in front.

Exercise 3. The Appendages About the Mouth

(i) Remove the labrum and clypeus, and thus expose the mandibles, the large jaws of the insect. Each mandible is a heavy, strong structure with black edges armed with teeth. The jaws of insects were evolved from structures corresponding to the legs. In the development of the insect, the jaws arise in the same manner as the legs, but are modified for a different use. It is for this reason that the grasshopper has right and left jaws, moving laterally, instead of upper and lower jaws as in the animals with which you are familiar. Separate the two mandibles a little at the tips with a needle or the point of a scalpel; and sketch the lower part of the face as seen from the front to show the mandibles. Remove the right mandible, by inserting the point of a scalpel between the gena and the base of the mandible. Sketch the appendage as seen after removal.

(j) Back of the mandibles are two other pairs of appendages: the **maxillæ,** a pair of more delicate jaws; and the **labium,** which serves as the lower lip. Each maxilla has a basal portion which bears three other parts: (1) a short feeler or **palp;** (2) the **galea,** a curved part with a rounded end; (3) the **lacinia,** a curved part which ends in some sharp, black

teeth. The labium has a basal portion, which bears two palps; and a flattened medial portion, which is partially divided into right and left halves. The labium is in reality composed of two appendages (right and left) which have become partly united in the median line. Compare in this respect with maxillæ, mandibles, antennæ, and walking legs. Remove the right maxilla by grasping it at the base with forceps and carefully pulling it away. Draw this appendage on the same scale as used for mandible. Remove and draw the labium.

Exercise 4. General Internal Structure

(k) Expose the internal organs by removing the tergum from thorax and abdomen in the following manner: Cut off the legs. With scissors cut through the exoskeleton along either side of thorax and abdomen just above line of spiracles, being careful not to cut deep enough to injure internal structures. Carefully remove the piece thus cut loose, and before discarding it, look for the **heart** which usually comes off attached to dorsal mid-line. If the thin mass of muscles which clings to the piece in the abdominal region is stripped by grasping with forceps at the posterior end and pulling forward, the heart will be seen as a delicate tube lying upon the dorsal surface of the muscles. How far can you trace the heart anteriorly? Cut off also the top of the head nearly as far down as the bases of the antennæ, being careful not to break the head from the body. Pin the animal down under water by two pins through the bases of the jumping legs, and keep it under water for the following dissections of the internal organs.

(l) In the thorax and anterior part of the abdomen the **digestive tract** is now exposed. In the posterior part of the body the larger portions of the reproductive organs occupy a dorsal and lateral position and hide the digestive tract. These organs are somewhat obscured by the lace-like **fat-body** which should be carefully picked away from the top of digestive and reproductive organs.

Exercise 5. The Reproductive Organs

(m) If the specimen is a female, the **ovaries** will be seen as a large mass containing many good-sized, elongated eggs. If it is a male, the **testes** will form a compact mass in the posterior part of the abdomen. In both sexes the ducts from right and left reproductive glands unite below the digestive tract and discharge through an opening at the tip of the abdomen. These features are not easy to study, and the reproductive organs may now be removed so that the digestive tract may be more fully exposed.

Exercise 6. The Respiratory Organs

(n) The respiratory organs of insects are air tubes called **tracheæ,** which open to the outside by means of the **spiracles,** which have been previously observed on the outer surface of the body. The tracheæ may be seen as silvery-white tubes on the surface of the digestive tract, if they contain air, but if they contain fluid they will be hard to see. Examine them carefully with a lens. Press the digestive tract to one side and find some of the larger tracheal tubes as they cross from the internal organs to the spiracles in the body wall. You may also see air sacs among the internal organs. Take a bit of muscle from one of the legs of the insect, mount on a slide in water, and study with the compound microscope. Notice the muscle fibers and the fine, branching tracheæ. Minute branches of the air tubes thus extend to all parts of the body, bringing the air directly to the cells. Compare the manner of distributing oxygen in frog and grasshopper. Make a drawing of part of such a preparation.

Exercise 7. The Excretory Organs

(o) On the surface of the digestive tract in the abdominal region notice the small, crooked tubes, which are likely to be so numerous as to form a felt-work. Use the lens to distinguish between these and the silvery-white tracheæ with which they are interlaced. These are the **malphigian tubes,** the excretory organs of the animal. Where do they connect with the digestive tract?

Exercise 8. The Digestive Organs

(p) The digestive tract has the following parts: the **mouth**; the **œsophagus,** which lies in the head and cannot be fully seen until later; the **crop,** an enlarged portion in the thorax; several pairs of pouches, the **gastric caeca,** which surround the gut and discharge a digestive juice into it just back of the crop; the **stomach,** which extends from the crop back to the place where the malphigian tubes join the tract; the **intestine,** from this point to the anus. The intestine has a more slender part, called the **colon,** near its middle; and terminates in a larger part, the **rectum.** Make a large drawing of the digestive tract as seen from the side, surrounded by an outline of the entire animal.

Exercise 9. The Nervous System

(q) Remove the digestive tract by cutting it across at the œsophagus and the rectum. The nervous system, which lies along the mid-ventral line. may now be exposed by picking away the other tissues. The nervous system consists of masses called **ganglia,** connected with each other by a longitudinal nerve trunk. Each ganglion gives off nerves to surrounding parts of the body. There are, in reality, ten pairs of ganglia, since the apparently single ganglionic masses are each composed of a right and a left ganglion fused together. Hence the nervous system consists of pairs of ganglia, united by transverse fusions, the **commissures,** between the members of a pair, and by longitudinal unions between successive pairs, the **connectives.** Nine of the ten pairs of ganglia lie along the mid-ventral lines close to the exoskeleton. The remaining pair constitutes the **brain,** which lies in the front of the head close to the bases of the antennæ sending large nerves to the compound eyes and smaller ones to the ocelli and the antennæ. Of the ventral ganglionic masses, five are in the abdomen, three in the thorax, and one in the head. The one in the head is termed the **sub-œsophageal** because of its relation to the œsophagus. It is hidden by skeletal structures and is very difficult to dissect out; but the connectives between it and the

brain, and between it and the first thoracic ganglion will be easily found. Such a nervous system is characteristic of the entire phylum to which the grasshopper belongs, and also of the segmented worms like the earthworms. Compare its position and structure with the nervous system of the frog or man. Draw the nervous system from a dorsal view, including an outline of the body and placing each ganglion accurately in its proper segment.

MISCELLANEOUS INSECTS

II. THE BEETLES.

Exercise 10. External Features

(a) Any large beetle will do for this study, provided it is not too highly modified. By examining the animal from the ventral side locate the **head, thorax** and **abdomen** and the number of segments visible in each. Look for **antennæ, compound eyes, ocelli, mandibles** and other **mouth parts,** the **anus** and the **thoracic legs,** and compare with what you have found in the grasshopper. Where are the **wing covers** and the **wings**? When the latter are found see how they fold up beneath their covers. Fasten down, dorsal side up, by pinning through the prothoracic segment, spread one wing cover out at right angles and unfold the corresponding wing which can be spread in the angle between the wing cover and abdomen. Raise the head, if it bends too far ventrally, and spread out the three legs on the side where wing and cover are closed. Draw the specimen from this view and on such a scale as to make the figure three or four inches long. Show the plates of the skeleton with care and number the segments of thorax and abdomen.

Exercise 11. Larval Stages

(b) In the larva of a beetle find the main divisions of the body, **head, thorax** and **abdomen; mouth** with its **jaws**; and the **anus.** Count the number of segments comparing with adult of the same species. Draw such a larva from a lateral view showing these parts on a scale of 3 or 4.

(c) Examine, as directed by instructor, such living specimens of beetles and their larvæ as are available for individual study or demonstration.

III. THE WASPS AND THEIR ALLIES

Exercise 12. External Features

(a) Wasps of the genus **Polistes** are very common and are easily collected when they enter unscreened buildings with the approach of cooler weather in the fall. **Head, thorax** and **abdomen** will again be recognized as in the case of the other insects. How many segments in each? Look for **antennæ, compound eyes, ocelli, mouth parts** and **anus.** At the posterior end of the female is the **sting.** The **spiracles** are a row of minute dots on each side of the abdomen. Compare the divisions of the thorax and of each of the **legs** with the corresponding parts of the grasshopper. To which segments are the **wings** attached? Draw a side view with wings spread dorsally, on a scale of 3 or 4.

Exercise 13. Nests and Larval Stages

(b) Examine the "paper" nests of this wasp and others if available. Also artificial **ant's nests** and the **eggs** and **larvæ** recently taken from an ant colony. The most remarkable facts regarding the hymenoptera are those connected with their social life in such colonies, a matter which will be discussed in lectures or textbook.

IV. THE BUTTERFLIES AND MOTHS

Exercise 14. External Features

(a) Examine a good-sized butterfly, or moth, going over the features noted for other forms (the three main **divisions of the body, eyes, antennæ, mouth parts, legs** and **wings**). Mount some of the dust from the wing surface and examine under a microscope. What is the significance of the term "lepidoptera"? Draw the entire animal from a dorsal view, with

wings spread, making the figure three or four inches across. Omit color pattern.

Exercise 15. The Life-cycle

(b) If available, the eggs of butterflies or moths will be shown as a demonstration. Understand to what species such eggs belong and where they are laid.

(c) Examine now a larva which is large and favorable for study. Where are the **head, thorax** and **abdomen**? Do you find **thoracic legs**? There are other pairs of appendages somewhat like them and known as **prolegs.** How many are there and what is their structure as compared with the thoracic legs? Are there **eyes, ocelli, antennæ** and **mouth parts** as in other forms? Do you find spiracles? Draw a side view on a large scale.

(d) If it is the proper season, the larvæ of various forms will be placed in the laboratory for individual study or demonstration. Observe the way of moving and their voracious habits in feeding. How does the structure and use of the mouth parts differ in larva and adult? At the proper season, caterpillars will often spin their coccoons in the cages where they are kept, or such coccoons may be collected and given out for study. Cut one open and find the resting stage, **pupa,** within. Notice the silk of which the coccoon is composed. Such coccoons if uninjured may be kept in cages and the emergence of the adult insect observed at some subsequent time.

(e) Understand the complete life-history in each of the groups thus far studied and be able to explain the difference between **direct development** as in the grasshopper and the **indirect development** or **metamorphosis** as found in the butterflies, beetles, etc.

V. OTHER ORDERS

Exercise 16. Examination of Museum Specimens and Special Assignments

(a) Of the remaining orders of the Insecta, three are more commonly known and recognized by popular names. These are: the **Hemiptera,** or true bugs; the **Diptera** or two-winged flies, of which the house fly is our most common representative; and the **Odonata** or dragon flies. Representatives of these and of their larvæ will be placed in the laboratory for demonstration and supplied to individuals if called for.

(b) The life-history and habits of insects presents much which is even more profitable for study than the points heretofore covered, but such work is difficult to handle properly with large classes and at fixed periods. Suggestions and assignments will, however, be made upon request to the instructor in charge and facilities for carrying on such work either at home or in the laboratory will be provided.

MITOSIS

CELL DIVISION

Exercise 1. Typical Mitotic Division

(a) The finer details of cell division must, of course, be studied with the very highest magnifications, but the more general features may be examined with the high powers ordinarily used in a course of this nature. **Mitotic** or **indirect cell division** appears to be the common method by which cells divide. The **amitotic** or **direct** mode of division seems to be of less importance and its significance is still a matter of doubt. For the study outlined below, sections of growing onion root tips or the epithelium of salamander larvæ may be used.

(b) Examine the sections with low power to understand the relation of the parts. Then with highest powers look for cells, in different stages of division, showing the **chromatin** in the

form of **chromosomes.** Examine chart or textbook diagrams of mitosis, and determine which phase of the process is represented by each cell found in division. Determine, if possible, **the number of chromosomes** in each cell.

(c) Construct six cell-outlines for figures showing consecutive stages. Then put in the details as you find good examples of the several steps in the process. Have the series in order when completed, but do not try to find them in this order. Rather, take representative cells as found in searching over the slide and draw into their proper place in the series.

(d) The following terms have come into use for designating the stages in cell division:

Prophase. The division and migration of the **centrosome** and formation of the **spindle,** the assumption by the **chromatin** of thread-like aggregates which segment into **chromosomes,** and the arrangement of the chromosomes into an **equatorial plate.**

Metaphase. The lengthwise splitting of the chromosomes.

Anaphase. The divergence of the chromosomes into the two daughter groups, and the division of the cytoplasm.

Telophase. The appearance of a **nuclear membrane** in each daughter cell, and the reconstruction of the nucleus to its typical **resting condition.**

(e) See that all the structures indicated above are properly labeled. Test your understanding of the spatial relations of parts by seeing whether you can readily interpret sections cut at irregular angles.

MATURATION AND FERTILIZATION

Exercise 2. The Maturation Divisions

(a) Demonstrations may be examined showing: (1) **polar bodies,** in superficial view; and (2) the **reduction divisions** of the **oöcytes** and **spermatocytes,** in sections. Understand the universal occurrence of this process and its relation to fertilization and the number of chromosomes.

Exercise 3. Fertilization

(b) Examine demonstrations showing the **entrance of the sperm** and the **conjugation** of male and female **pronuclei** to form the **cleavage nucleus** in the egg of an echinoderm or other favorable material.

DEVELOPMENT OF ECHINODERMS

Exercise 1. The Cleavage, Gastrula and Blastula Stages

(a) The eggs and spermatozoa of many marine animals are laid directly into the water where they meet in fertilization. Such eggs are usually small, having but little yolk. They develop rapidly into feeding larvæ, which swim for a time, and then take up the life of the parent upon the bottom. Because of the ease with which they can be provided with their normal environment, these eggs are particularly favorable for experimental studies and have become classic material for the study of fertilization, artificial parthenogenesis, cleavage, and the like.

(b) Examine stained material permanently mounted or in clearing fluid, showing **cleavage, blastula** and **gastrula** stages in the egg of the starfish or sea-urchin. Note the egg membrane, sometimes showing the heads of many spermatozoa which failed to enter. The **two-, four-, eight-cell,** and later **cleavage stages** on to the **blastula** or hollow sphere stage, will be recognized. Find stages of the blastula, showing the ingression of **mesenchyme** cells at one pole. Is the wall of the blastula of uniform thickness? Can you recognize, even during the blastula stage, the region that will **invaginate** to form the next stage, the **gastrula,** in which the primitive gut cavity or **archenteron** is formed? Its opening is the blastopore. The process of invagination is termed **gastrulation.** The **germ-layers, ectoderm** and **endoderm,** have now been formed. The mesenchyme cells noted above and other cells which arise from the blind end of the archenteron constitute the **mesoderm.** Make a series of outline figures illustrating the foregoing. Size about two inches in diameter.

Exercise 2. The Larva and its Metamorphosis

(c) As development proceeds, the blastopore of the gastrula becomes the **anus** of the larva. The **mouth** is formed by an invagination, the **stomodæum,** which unites with the blind end of the archenteron. Examine demonstrations. A **larva** which is strikingly bilateral results. From this, by a curious **metamorphosis,** the radially symmetrical adult is formed. The existence of such a larva constitutes the main evidence for the belief that the present radially symmetrical echinoderms have descended from bilaterally symmetrical ancestors. Compare with the inferences drawn from the existence of fish-like stages in frog and chick.

THE DEVELOPMENT OF THE LEOPARD FROG

I. BREEDING HABITS

Exercise 1. Collection and Study of Living Material

(a) The frogs and salamanders lay their eggs in spring and early summer, the period of laying being as definite a characteristic of the species as any other form of behavior. If this study is made during the laying season, go out and collect eggs for yourself, examining the breeding places and studying the activities of the animals under natural conditions. Are there any easily recognizable differences between males and females? Do frogs croak or "sing" more at this season? Why?

(b) Take home a mass of living eggs you have yourself collected or have obtained from the laboratory. Place in a shallow dish and keep in a well-lighted place, but not exposed to direct sunlight for much of the day. Record the stage of the eggs when obtained and note their progress from day to day. Preserve your notes in the form of a written report to be handed in later. The influence of temperature upon the rate of development can be tested by placing part of the eggs out of doors on the cool north side of a building and comparing them each day with those having sun and the warmth of in-

doors. With proper care the animals may be kept until the tadpoles have completed their metamorphosis. At no time should the water in the dish be allowed to become too low from evaporation or to become foul from the growth of bacteria. Green water plants will be beneficial, unless growing in too dense masses. When the tadpoles are fully formed, they may be fed upon bread or cracker crumbs, but too much of this food will foul the water and care must be used.

(c) Examine living frogs, toads, and salamanders in the laboratory or field. Make a table giving common and scientific name of each species and the characteristics of its egg mass.

II. THE UNFERTILIZED EGG OR OVUM

Exercise 2. The Ovarian Egg

(a) Examine in a watchglass of water a small mass of eggs from the ovary of a frog preserved in formalin. Look with handlens and lowest power of compound microscope for the smaller eggs among the larger ones of the present season. Some of the former will show, when examined under low power of the compound microscope, a **nucleus** and a small amount of **cytoplasm.** The larger eggs are opaque and their internal structure cannot be seen in this material. They are single cells like the smaller ones; but have grown large, as a result of the yolk material which is laid down in their cytoplasm. The egg in these stages before fertilization is termed the **ovum.** Draw one or more of these smaller ova, showing the parts in outline only.

(b) Examine a permanently mounted and stained section of the ovary in a young frog. In addition to the structures observed in (a), this will show the **follicle cells** surrounding each ovum. Add these to your drawing.

(c) Remove a single large ovum from the ovary of a recently killed frog; and, after crushing on a slide under a cover slip, examine with a high power. What is the physical nature of the **protoplasm**? Observe the numerous **yolk bodies.**

Can you discover any structure which foreshadows that of the tadpole or frog? Record or draw.

III. THE SPERMATOZOON

Exercise 3. The Sperm Cells within the Testis

(a) Spermatozoa may be secured by cutting a testis into small bits and teasing in water. Examine the fluid under compound microscope; and look for elongated bodies, the **sperms** or **spermatozoa.** Each consists of an enlarged portion containing the **nucleus,** and an elongated thread-like **flagellum,** Do the sperms move about? During the breeding season active or "ripe" sperms will be found. As in the case of the ovum, the spermatozoön is a single cell. Draw one or more spermatozoa. Size about three inches in length.

IV. THE EGG-LAYING AND FERTILIZATION

Exercise 4. The Fertilized Egg or One-cell Stage

(a) Recall the exact structure of the male and female reproductive organs as dissected in the adult, and the manner in which the ovum passes from ovary to oviduct. The **jelly,** which is so conspicuous a feature of the masses of eggs seen in ponds, is secreted around each ovum during its passage through the oviduct. Fertilization occurs in the water outside, either as the eggs leave the cloaca of the female and while the animals are still joined in copulation or, at latest, before the swelling of the jelly which occurs during the first few hours of exposure to the water.

(b) Examine several eggs which have the jelly well swollen. Is the jelly arranged in layers? These and the subsequent stages should be studied in a watchglass with enough water to cover them. Use the handlens mainly, and only in special cases the lowest magnification of the compound microscope, turning off the mirror. When it is desirable to remove the jelly, from preserved material, this may be done by rolling the egg along on a piece of filter paper. In the living egg,

the light colored hemisphere is the heavier; and since the egg turns readily within its envelope of jelly the darker surface is always uppermost. How does the color on the surface of the egg compare with the general distribution of light and dark color on adult frogs, fishes, birds, etc., with which you are familiar? What significance may this have? The center of the darker or pigmented hemisphere is called the **animal pole,** the opposite point on the sphere the **vegetative pole** of the egg. Draw several eggs (x 3), showing their envelopes of jelly as they lie together in the mass; also a single egg from a side view, one inch or more across, to show the pigmentation and the layers of the jelly. Label the poles and indicate the polar axis by an arrow, drawn as though thrust through the egg.

V. THE DEVELOPMENT OF THE FERTILIZED EGG

(a) It is desirable that the cleavage and later stages described in the directions which follow be studied in living material. But since this is obtainable only in the spring, these directions have been written with reference to preservd material. In studying this dead material, examine not only the exact stages specified, but also look for intermediate conditions and wherever possible arrange specimens in a series showing the exact transition from one stage to another, thus obtaining a picture of the development which approaches the vivid impression conveyed by the living egg and embryo as it passes from one stage to another.

THE CLEAVAGE STAGES

Exercise 5. The Two-cell Stage

(b) In preserved material this stage is best studied in an egg with the cleavage furrow encircling about two-thirds of the circumference. Examine several specimens in a watch-glass, using handlens and low power of compound microscope. In the living egg, this **first cleavage furrow** begins at the animal pole and gradually extends around the egg. When the furrow has cut entirely through, as well as around, the egg the two-cell stage is complete.

(c) Understand that after the entrance of the spermatozoön in fertilization the nucleus of the sperm unites with the egg nucleus to form a single cleavage nucleus. The two-cell stage begins with the division of this single nucleus and the cleavage furrow seen on the outside is only the final step in the cell division by which the one-cell stage divides to form the two-cell. Following the completion of the first furrow a short period ensues during which no external changes occur, but internally the nuclei are preparing for the next cell division.

(d) Draw from side and top views to show the first furrow in process of formation, or as it appears when completed. Indicate the poles by an arrow as before, and number the furrow by a figure 1 at either end. The most satisfactory size for the egg will be a circle 1½ to 2 inches in diameter, a scale which should be continued in subsequent drawings. Do not represent the jelly in this or subsequent drawings unless the directions call for same.

Exercise 6. The Four-cell Stage

(e) Examine several preserved specimens of eggs in which the **second cleavage furrow** is well advanced. What has happened internally before this furrow appears on the surface? Draw side and top views of this stage, numbering the furrows 1 and 2, and labeling the poles as above.

Exercise 7. The Eight-cell Stage

(f) The **third cleavage furrow** is horizontal. How is it placed with reference to the equator of the sphere? With its completion, we have the eight-cell stage which has four smaller, deeply pigmented cells above and four larger, lighter colored cells below.

(g) Understand that with each division of any one cell, the nucleus also divides; so that in the two-, four-, eight-cell, and later stages, and so on to the many-celled adult organism, each cell possesses a nucleus descended through a longer or shorter series of divisions from the original nucleus of the one-cell stage or zygote which was itself formed by the fusion

of nuclear material from egg and spermatozoön. Hence, we reach the generalization that every cell of the adult animal contains a nucleus descended one-half from the male and one-half from the female parent. Draw top and side views of this stage, indicating the poles and furrows as before.

Exercise 8. The Twelve- and Sixteen-cell Stages

(h) The **fourth cleavage** consists, theoretically, of two vertical furrows which appear simultaneously at right angles to one another, dividing each of the eight cells approximately in halves. The four upper cells often complete this division before the furrows have appeared in the lower hemisphere; thus making, with the eight smaller above and the four larger cells below, a twelve-cell stage. With the division of the four lower cells, the sixteen-cell stage is produced. Examine a number of eggs preserved in this stage and determine the exact outlines of the cells at either pole. What forces determine the cleavage pattern at this stage? Draw, as before, a twelve- or a sixteen-cell stage from top and side views, reproducing accurately the cell outlines of a typical specimen.

THE BLASTULA AND GASTRULA STAGES

Exercise 9. The Thirty-two-cell Stage or Early Blastula

(i) Theoretically, the **fifth cleavage** consists of two horizontal furrows, one above and one below the third. Which of these would you expect to appear first if you were studying the living egg? With the completion of these furrows we have a **thirty-two-cell** stage. Why is it difficult in studying any single preserved specimen of this stage to assign numbers to the different furrows? A thirty-two-cell stage is a theoretical rather than an actual occurrence, because the cells about the upper pole divide faster than the yolk-laden cells of the lower hemisphere. Why is there this difference in the rate of division? In the living egg the development is continued by progressive cell divisions, but from this time on it is impossible to recognize any uniformity in the pattern made by the fur-

rows. The term "mulberry stage" is often applied to this stage in which the number of cells becomes so great that the order of the furrows can no longer be traced. More definitely, we shall speak of it as the **early blastula stage,** the complete blastula stage being reached only by the further subdivision of the cells. Draw a theoretical side view of the thirty-two-cell stage, numbering poles and furrows; and beside this draw a side view showing the exact appearance of an egg which is in about this stage of development.

Exercise 10. The Blastula in Section

(j) Take a preserved specimen in an early blastula stage; and after removing the jelly by rolling on damp filter paper, divide it into halves by a vertical cut with a sharp scalpel. Several trials may be necessary, but remarkably good preparations are often secured by this rough method. Study under water with handlens and low power of compound microscope with mirror turned aside. What is the extent of the internal cavity? What is the size of the cells? Have the cells begun to divide in planes parallel to the surface of the sphere? This **cleavage** or **blastula cavity** is a characteristic feature of the blastula stage. In the amphibian egg it appears at about the twelve- to sixteen-cell stage and persists until obliterated by the development of another cavity which becomes the digestive tract of the adult. Draw a favorable section to show these features.

Exercise 11. The Late Blastula

(k) Examine stages some hours older than the above and showing surface cells of much smaller size. Is there any sign of differentiation anywhere on the spherical mass other than the difference in pigmentation? Draw from a side view, showing size of cells by making a few cell outlines at either pole, labeling poles, and indicating distribution of pigment.

Exercise 12. The Early Gastrula Stage

(l) Examine a stage several hours older than the last, using handlens and low power. Can you recognize cell outlines at each pole? If a specimen is not found with the vegetative pole up, one may be secured as follows: cut a piece of filter paper one-half inch square and lay upon a slide; add enough water to saturate and hold the paper in place but not enough to float it; using another piece of the paper, roll off the jelly from a single specimen and transfer to the above slide. The specimen may then be rolled into the desired position. If details are not clear under low power, add coverslip, supported on one side by a pin, and run water under the cover with pipette. Examine the vegative hemisphere and the equatorial region. At this stage the dark cells of the animal pole begin to encroach upon the surface area of the lower, light colored cells. The exposed surface of the lighter cells thus becomes diminished around its entire margin by the overgrowth of the darker cells. This overgrowth of the darker cells begins on one side of the equatorial region as a crescent-shaped line separating the light and dark cells. In later stages the crescent becomes extended to form a circle. Does the gastrula stage, as here studied, exhibit a bilateral symmetry which may foreshadow that of the future adult? The crescent-shaped line between the light and dark cells is known as the **blastopore**; and is, in reality, a narrow slit which leads into the developing gut cavity. This stage is called the "gastrula" because the gastric or digestive cavity is now being formed. Draw the early gastrula, as seen from the vegetative pole.

(m) Understand from lectures or textbook the internal changes by which the blastula becomes a gastrula. The word "egg" has been applied to the developing organism up to this point, although strictly speaking the egg, having started on its development, ceases to be an egg and becomes an "embryo" even in the two-cell stage. The term "ovum" is invariably used to designate the egg cell before fertilization, but the term "egg" is more loosely used; and embryologists commonly speak of the developing egg until an embryo is formed which

is no longer egg-like in appearance. During its blastula and gastrula stages, the frog undergoes this change, and we shall accordingly begin to use the term "embryo" at this point in the development.

Exercise 13. The Late Gastrula Stage

(n) Examine an advanced gastrula stage in which the dark cells have still further encroached upon the exposed area of vegetative cells. This is often called the **"yolk-plug" stage,** because the yolk laden vegetative cells appear as a small "plug" on the otherwise dark colored embryo. Make a simple outline drawing, oriented and labeled in a way to be understood when compared with previous figures.

(o) Examine a demonstration showing the **gastrula cavity** or **archenteron** growing in from the **blastopore** and the earlier **blastula cavity** being obliterated. Cut a gastrula into right and left halves and examine as in the case of the blastula. Good selections will show the cavities as clearly as in the demonstration specimen and have the added advantage of showing the third dimension. Understand from lectures and textbook the internal structure of the gastrula, i. e., its **ectoderm, endoderm, mesoderm** and **archenteron**; and how the bilateral symmetry of the embryo is made evident during gastrulation. The yolk-plug finally disappears within the embryo. But the blastopore can still be distinguished as a minute pit, which is to be shown in drawings of the stage immediately following. Draw the section studied, oriented in such a way as to place the future dorsal region toward the top of the page. The future head region may be to the right or to the left, but in subsequent figures the orientation here chosen must be continued.

THE NEURAL FOLD STAGES

Exercise 14. The Early Neural Fold Stage

(p) Examine stages some hours older than the last. The yolk-plug has disappeared by overgrowth of the dark cells. The ectoderm has thickened along the future dorsal region,

and the right and left **neural folds** are seen as slightly elevated ridges bordering this dorsal area. How are the right and left neural folds related to one another at either end? Find the blastopore, which now lies at one end of the area enclosed by the neural folds. Although this stage is not greatly changed from the spherical condition immediately preceeding it, we can now clearly recognize the parts of the adult body as follows: The bilateral symmetry is obvious; the region between the neural folds is on the **dorsal mid-line**; the opposite surface is, of course, the **ventral**; the end where the folds are more widely separated represents the **anterior**; the end where the blastopore is located the **posterior region** of the future adult. What is the position of the blastopore with reference to the neural folds? Draw this stage from a dorsal view, making outline of the jelly still surrounding, and labelling to show all the above points in their relation to the future adult. The jelly begins to disintegrate at about this time and the preserved tadpoles are likely to become separated from it before the time of hatching.

Exercise 15. The Late Neural Fold Stage

(q) Examine a later stage. The neural folds have become higher and approached one another along the dorsal mid-line. Where do they fuse last of all? What has become of the blastopore? What general changes in the shape of the entire body have taken place? Understand from lectures and textbook what develops from that part of the ectoderm which is folded in when the neural folds meet and fuse, also the structures which would appear in longitudinal and transverse sections of such a stage. Draw a side view with the ventral surface below, labeling thoroughly.

THE EARLY LARVAL STAGES

Exercise 16. The First Tadpole Stage

(r) Examine stages in which the shape of the tadpole is beginning to appear. Several specimens of slightly different

ages are desirable, as the earliest rudiments of parts are not easily recognized. Determine first the orientation. The dorsal line is less curved than the ventral which protrudes in the belly region. Where is the line of fusion of the neural folds? The head end is blunter than the tail at this stage. In the region between body and head, there is on each side a slightly raised area, the **gill plate.** Anterior to this is the rudiment of the sucker, recognizable as a "U" shaped structure when seen from the ventral aspect. Examination of the posterior end will show the **proctodæum** or rudiment of the anus. The specimen should be rolled over and studied from different angles, or several specimens in different positions should be examined. Draw a side view, orienting in the same way as the last figure. Making a model may prove helpful.

Exercise 17. The Second Tadpole or Hatching Stage

(s) The term "larva" is applied to a stage in an animal's development, structurally different from the adult and leading an independent existence. As previously explained, the term "embryo" is applied to stages preceding the larval stages, when the latter are present; and embryos are, in general, unhatched stages. How would you apply these terms to the development of a butterfly, a bird, and a mammal? The tadpole is said to "hatch" when it frees itself from the jelly and begins to swim about. Examine specimens in this stage. The head, body, and tail are becoming evident to the unaided eye. The tadpoles are leaving the jelly, which has become very soft, and preserved specimens are often shaken free from the jelly before this stage. The specimens given out will probably be in slightly different stages and if a half dozen are taken they can be oriented alike and arranged in order to show the developmental changes. Study such a series and follow the further differentiation of **the parts shown in the last figure.** The rudiments of the eyes will now be seen on either side near the most anterior portion of the body.

(t) Select a stage showing clearly: the head, body, and tail, the rudiments of gill-plates, the eyes, the suckers, and the

proctodæum or future anus. Draw, orienting as above. Here again, modelling is helpful.

(u) Cut cross sections through the body, placing the specimen on wet filter paper and using a sharp scalpel as with the blastula and gastrula stages. Good sections will show: the tubular **nervous system,** the primitive backbone or **notochord,** the gut cavity lined with **endoderm,** the **yolk mass,** the **mesoderm,** and the **ectoderm.** Compare with chart or other figures and identify these parts as far as possible, making a diagrammatic figure of such a section.

Exercise 18. The Third Tadpole Stage

(v) Select a number of specimens which can be seen with the eye to have the tail beginning its differentiation into a **fin** and a muscular **axis.** Arrange a series, as before, and after rejecting duplicates, rearrange the series changing to clean water if necessary. At one stage the larva will show clearly certain segmental markings on either side in the region of the backbone. These are the primitive **muscle segments.** Identify **all the parts previously studied;** and, in addition, the rudiments of the **external gills,** appearing as a tuft-like growth on either gill plate; and the **tail fin.** Draw, orienting as before.

Exercise 19. The Fourth Tadpole Stage

(w) Up to this time the tadpole, although hatched, swims but little. Instead, it clings to the jelly and other foreign bodies by means of the suckers. Now the activity becomes greater, and in the living specimens swimming is much more in evidence. Study specimens with well developed external gills, **identifying all the parts as above;** and in addition, the rudimentary mouth or **stomodæum,** which now appears as a pit anteriorly between the suckers and eyes. It is clearly seen from the ventral view. Where is the proctodæum at this stage? Parts of the **brain** show through the ectoderm as swellings above the line between eye and gills. Look for muscle segments. What is the distribution of the pigmentation

at this stage? What correlation exists between the appearance of the mouth, the disappearance of the yolk, and the assumption of an active mode of existence? Draw, orienting as before.

Exercise 20. The Fifth Tadpole Stage

(x) In individuals more advanced than the foregoing, look for the **operculum,** a membrane comparable to the lateral covering of the gills of a fish. It will be found overgrowing the external gills. The external gills are temporary respiratory organs and soon disappear with the growth of the true gills, which are in the region covered by the operculum and are comparable to the gills of a fish. Draw a tadpole, showing the external gills partially overgrown by the operculum.

THE LATE LARVAL STAGES

Exercise 21. External Features

(y) For this study preserved tadpoles of the leopard frog or of the large bullfrog may be used. Living specimens should be examined in an aquarium. Notice how they come up to breathe and how the more advanced stages are beginning to use their legs. Study first a preserved specimen with hind limbs just appearing. It should be placed under water in a dissecting pan. Make out: **nostrils; eyes; mouth,** with horny **teeth; hind limbs;** and, on one side of the body, the **opercular opening** which leads into the **gill chamber.** Draw from a side view, orienting as in previous figures, and showing the opercular opening by a dotted outline if your drawing is of the right side.

Exercise 22. The Gills and Viscera in Position

(z) Fasten the specimen ventral side up, by pinning through mouth and tail. Remove the skin from the ventral half of the body wall, leaving the opercular opening intact. The coils of the **intestine** may now be seen beneath the muscles and just anterior to them the gill region. Are there any indica-

tions of segmentation? Carefully remove the thin layer covering the gills, and find the **fore limbs** lying against the gills and within the **opercular cavity**. Probe through the opercular opening and determine its relation to the gills. Find the **heart** between the right and left gill areas. Anterior to the gills are three conspicuous **muscle bands** connected with the jaws. Remove the muscles which cover the coils of the intestine. How are the cœlomic and opercular cavities separated?

(aa) Pin out beside the tadpole and in the same position a small fish, preferably a catfish. Examine the gills and the operculum of either side, if necessary cutting anteriorly from the "V" where the two opercula meet. What is the relation of the gills and the gill slits to the mouth and to the cavity beneath the operculum? Locate the heart by cutting along the mid-line in the angle of the "V".

(bb) Compare these structures in the tadpole and the fish. How many gills and how many gill slits in each? What is the relation of gill slits to mouth in the tadpole? Using a tipped bristle, probe through the mouth and out through the gill slits. Recall the earlier stages of the operculum as seen in Exercise 20. How does the operculum of the tadpole differ from that of the fish? Is the developing fore limb really on the outside of the body?

(cc) Draw the entire tadpole (x 2), as thus dissected showing: the cut edge of the body wall; the partition between cœlome and opercular cavity; and the other structures observed. Show the relation between opercular cavity, gill slits, and mouth by arrows. Spread the gills apart to show the slits.

Exercise 23. The Cœlome and its Contents

(dd) Lift up the mass of the intestine at its posterior margin and locate the **rectum** against the dorsal wall on the left side of the body. Cut the rectum leaving a short stump. Lift the mass of the intestine and find the **œsophagus** where it enters the anterior end of the cœlome. Follow the œsopha-

gus to the **stomach** and this to the intestine where the latter enters the coil. Cut the intestine at this point and remove the coil after cutting its attachment to the dorsal mid-line. Look for the **spleen,** either on the dorsal side of the coil as removed or still in the body. Uncoil the intestine and determine its length. How does it compare with length of the intestine in the adult? Is there a large intestine? Is there anything in the feeding habits of frog and tadpole which may explain the different length of the intestine in the two? Examine the part of the digestive tract remaining within the cœlome. Find, in addition to the parts above noted, the **liver** with the **gall bladder** between its lobes, and the pancreas lying in an angle between the stomach and the intestine. What changes must occur in the digestive tract when the tadpole becomes a frog? Locate the **lungs, kidneys, fat bodies,** and the rudiments of the **ovaries** or **testes.** Draw the above organs as they appear in position or slightly displaced to show as much as possible, making an outline of the tadpole and its cœlomic cavity about the organs.

Exercise 24. Transverse Sections

(ee) Unpin the specimen and with a sharp scalpel cut transversely through the body in the gill region and again at about the middle of the cœlome. What is the condition of the skeleton and of the nervous system in comparison with the adult as shown by the two sides of the thick section? If not clear, take a new specimen and again cut sections. Draw a favorable section or sections.

THE METAMORPHOSIS

Exercise 25. The Tadpole of a Frog

(ff) Examine several preserved specimens showing stages in the metamorphosis. Note changes in shape of body, in mouth, tail, limbs, and coloration. What becomes of the operculum? Has the tympanum appeared? Draw representative individuals.

Exercise 26. The Tadpole of a Salamander

(gg) During the spring months the living tadpoles of the salamander Amblystoma may be obtained. Watch individuals, sometime hatched, as they swim in an aquarium; and then examine, in a watchglass, with a handlens. Compare part by part with the tadpoles of the frog already studied. The suckers are long stalk-like structures, otherwise the resemblance is obvious. Add a drop of ether to the water and when the larvæ become quiet examine with low power of compound microscope. Note pigmentation of the body. Observe the blood-flow in the gills. Can you recognize corpuscles? Pulse? Construct a diagram to show the course of the blood-flow in a single tuft of the gills.

(hh) Older larvæ are often taken during the early summer in places where frog tadpoles are abundant. Examine preserved specimens about one and one-half inches in length and showing external gills just disappearing beneath operculum. Compare with demonstration specimens of the adult. Can you recognize muscle segments in larva and adult? What structures justify the statement that a salamander is a less specialized vertebrate than a frog? Draw the larva from a side view, orienting the same as figures of the frog tadpoles.

THE DEVELOPMENT OF THE CHICK

Exercise 1. The Reproductive Organs

(a) Examine the reproductive organs of a male and those of a laying female: to see the **testes,** and their **vasa deferentia:** the **oviduct,** with its **funnel,** its **albumen-** and **shell-secreting** parts, and its relation to the **cloaca** and **rectum.** Notice, in the **ovary,** the **eggs** in various stages, and the places where eggs have been recently discharged; also the **stigmata** or non-vascular areas which rupture when an egg is set free.

(b) Examine under the microscope a small amount of the **yolk** obtained from one of these ovarian eggs. Recall the similar structures in the frog's egg.

(c) Examine demonstration sections showing the cellular nature of the ovary.

Exercise 2. The Unincubated Egg

(d) Take an **unincubated** hen's egg and, using scissors, cut open on one side a space about one inch across, being careful that the scissors points do not cut too deep and injure the yolk. The opening may be further enlarged, if necessary, the egg resting upon a bed of cotton wool in a finger bowl. Find the **chalazæ** or twisted cords of albumen at either end. What relation have they to the yolk and to the shell? Find the two **membranes** which line the shell. These can always be seen at the large end where there is a space between them. At one place upon the surface of the yolk is a small whitish area, the **blastoderm,** the central part of which is known as the **area pellucida** and the peripheral part as the **area opaqua.** Does this always appear at the top, however the egg is turned? Compare with the rotation of the frog's egg in its capsule. Understand the comparison between such an egg as this and that of the frog and the starfish, and the condition of the blastoderm at this stage. Draw (x 1) the egg as thus dissected.

Exercise 3. The Twenty-four-hour Stage

(e) Open an egg which has been under incubation for **twenty-four hours,** and placing it on the cotton beside the one just drawn compare the two. Record or make a simple sketch, to show the changes which have taken place in the blastoderm during this first day of incubation. Before discarding this specimen, the existence of a delicate **yolk membrane** should be demonstrated by puncturing.

(f) Permanently mounted specimens of the blastoderm and the developing embryo will be issued for the study of approximately the 24-, 36-, and 48-hour stages, in their finer details. These should be handled with great care lest they be crushed by wiping or by the objectives of the microscope. These slides are secured by removing the blastoderms, which are then fixed,

stained, and mounted in balsam. The first to be studied is the **24-hour stage,** in which the following parts are to be made out with the low power of the compound microscope: **neural folds; head folds; mesodermal somites; primitive streak; area pellucida;** the **vascular area;** and the **vitelline area.** Focus carefully to determine the vertical dimensions of the parts and compare your results with what is shown by models. Draw this stage as a full-page figure, including a small margin from the vitelline area.

Exercise 4. The Thirty-six-hour Stage

(g) Open a **36-hour stage,** just from the incubator, and notice the further changes. With the aid of an instructor, inject some India ink into the cavity beneath the blastoderm, and then harden the embryo by dropping strong alcohol upon the outside. Compare part by part with a permanently mounted specimen of the same stage, placing the latter across the top of a watchglass and against a white background. Study with lens to locate **the parts previously observed in the 24-hour stage.** After identifying these with the handlens in both the fresh and the preserved specimens, study the mounted specimen further under the compound microscope and make out, in addition to the features seen in the last: the beginning of the **brain vesicles;** the **amnion; heart; notochord;** and any changes in the size and proportions of parts. Here again, careful focusing and the comparison of what you see with the models is necessary for the proper understanding of the third dimension. Draw this stage in a figure similar to the last.

Exercise 5. The Forty-eight-hour Stage

(h) Examine a freshly opened embryo in the **45- to 48-hour stage,** comparing it with the last. Note: the **blood vessels;** the **pulsations of the heart,** which should be counted for the number per minute; and the extent to which the blastoderm has extended over the egg. Treat with India ink and alcohol as before. Study the specimen thus freshly prepared

and a stained and mounted specimen of the same stage. Find all the structures previously obsurved in the 36-hour stage, and in addition note: the **cranial flexure**; the **torsion** of the cephalic end of the embryo; the **fore-, mid-,** and **hind-brain vesicles**; the **optic vesicles**; the **lens** of the **eye**; the **auditory vesicles**; the tubular **heart,** now bent into an "S" shape; the **vitelline arteries** and **veins** and the **sinus terminalis**; the **gill bars** and **slits**; and the extent to which the **amnion** has developed. Draw this stage, in a figure similar to the last.

Exercise 6. The Embryonic Membranes

(i) Understand from demonstrations of the later stages of chick and mammal and from the lectures, charts and text-book, how the embryo is related in its several stages to the yolk mass and the significance of the **amnion, allantois** and **yolk-sac** in birds and mammals.

THE CRAYFISH

PHYLUM, ARTHROPODA. CLASS, CRUSTACEA

I. BEHAVIOR AND EXTERNAL FEATURES

Exercise 1. General Activities

(a) These directions have been prepared with special reference to the crayfish **Cambarus virilis.** They may be used equally well for any of the common species of crayfish and, with slight modifications, for the lobster **Homarus americanus.** Watch the crayfish in shallow pans or aquaria and study their manner of swimming and walking. How sensitive are they to touch? By passing your hand a short distance above the specimen, see if it shows any sign of an acute sense of sight. Try this again with a specimen out of water. Place a specimen on its back in the water or on a table and determine the acuteness of its sense of equilibrium.

(b) Put some carmine in the water and see if you can detect any currents flowing in a definite direction in the vicinity of the animal when it remains quiet for a short time. Selecting a small individual, or one having a very clean shell, look on the outside of the body, just above the great claw and in line with the eye, and see if you can detect a flickering motion, as though something were moving beneath the semi-transparent shell. Recall this observation later when you come to study the gill chamber. Keep a specimen out of water for a few minutes and note the bubbles which come from the front part of the body when it is again placed in the water. Do they come from a definite place on the body? You should be able to give an intelligent explanation of the facts noted in this paragraph after you have studied the gill chambers of the animal as outlined in section II of these notes.

(c) Observe crayfish as they remain undisturbed in aquaria containing stones or other objects. Can you tell what determines the particular places which the animals occupy. Drop small pieces of fresh meat into the aquaria and watch the re-

sult. In some of the aquaria several small fish may be placed and the result watched at this and the next laboratory period. Observe crayfish in large tanks or in nature and note any habits of concealment and also their mode of swimming.

Exercise 2. External features

(d) The body of the crayfish is made up of **segments** or **somites** each one of which bears a pair of jointed **appendages.** There are three general regions of the body, **head, thorax,** and **abdomen,** each of which includes several segments. In the abdomen the segments are distinct and movable while in the remainder of the body they are covered dorsally and united by the **carapace** into a rigid portion, the **cephalothorax.** Compare anterior and posterior ends, dorsal and ventral and right and left sides. Is there any departure from strict bilateral symmetry? Examine the pairs of limbs from the anterior to the posterior end. Is the entire animal covered by a dense **shell**? What is the nature of the joints of body and appendages? After looking carefully at the proportions of the abdomen, draw (x 1) an outline of an ideal cross section through this region to show the shape of the dorsal and ventral parts of the shell and the shape and attachment of the paired limbs. Do not actually cut across the specimen, but make the figure as it would appear if cut across.

(e) Find the **mouth** and **anus.** Note the openings on the bases of the longest feelers, **second antennæ.** They are the openings of the kidney-like **green glands.** Find on the dorsal face of the small first antennæ the clear flat areas which mark the position of the **lithocysts** or organs of equilibration. Examine the bases of the walking legs for the openings of the reproductive organs. They are found on the last pair in the male, and on the second from the last pair in the female. In the male of **C. virilis** the two first pairs of abdominal appendages are modified to form, when pressed together, a **copulatory organ** along which the sperms pass after leaving the male openings. What is the condition of the corresponding appendages of the female? Place a male and a female side by side

and note the difference when they are viewed dorsally.

II. THE RESPIRATORY ORGANS

Exercise 3. The Gills and Respiratory Currents

(a) Note how the shell, carapace, extends from the back down over the bases of the walking legs. Lift up the free ventral edge and see the spongy mass formed by the **gills** or **branchiæ.** Taking care not to injure the gills and using your strong scissors, remove the overhanging shell from the left side, thus exposing the full extent of the **gill cavity,** but do not cut too far dorsally and injure the organs on that side of the body. Cut off the walking legs and large claw, **chela,** of this side a short distance from their insertion. Place the specimen under water in a dissecting dish and by floating up and carefully parting the mass of the gills get an idea of what a single gill is like and where it is attached to the body. Move the stumps of the legs and see how the outer gills are related to them. How many of these outer gills are there? To what appendages are they attached? What effect do you think the animal's walking would have upon respiration? What structure do you find at the place where you saw the flickering movement under the shell of the living specimen? Back of this **bailer** is another, more delicate, blade which you will identify later as the **epipodite** of the first maxilliped.

(b) Put together all you know about the gill cavity, its contents, and the water currents you have seen in the vicinity of a quiet animal, and explain how the gills are always bathed with a constantly changing supply of water.

(c) These outer gills are called **podobranchs.** Note the significance of the name. Keeping the specimen entirely under water and lifting the podobranchs one at a time to be sure you do not destroy any of the smaller gills which lie close beneath, remove all of the podobranchs by cutting them off close to their attachment. Cut one across the middle with scissors and examine the section under water with a handlens.

You should see the **incurrent** and **excurrent blood vessels** cut across where they run close together.

(d) The inner layer of gills is now exposed. Are they attached to the feet? There are five pairs and a single one in front. Opposite which of the appendages are these gills located? They are called the **arthrobranchs** (joint gills). Note again the significance of the name.

(e) In the lobster there is another layer of four gills lying beneath the arthrobranchs. Because they are attached higher up and on the sides of the body these last are termed the **pleurobranchs** (side gills). The common European crayfish from which the descriptions in most textbooks are taken possesses a single pleurobranch, but in the adult of **C. virilis** even this has disappeared. Examine museum specimens of the lobster dissected to show the three kinds of gills.

(f) Make an outline (x 2) of the cephalothorax in a side view. Show the stumps of the appendages and the places from which podobranchs have been removed. Put in all the arthrobranchs and show also the bailer and the epipodite above noted. Indicate the course of the water current by arrows.

(g) Examine specimens macerated in caustic potash and notice the delicate chitinous covering of the gills which has survived the maceration. Are the gills inside or outside the body? In answering this question, imagine how they would look in a cross section of the animal in the thoracic region of the body.

III. THE INTERNAL STRUCTURE

Exercise 4. The Digestive, Circulatory, and Reproductive Systems

(a) Using a freshly killed specimen, cut with large scissors along the dorso-lateral surface on either side of the cephalothorax, taking care not to injure any of the organs lying immediately beneath the skeleton. Remove this dorsal part of the skeleton from the posterior margin of the thorax to a point

just back of the eyes. Place the specimen in a dissecting dish and having it entirely covered with water identify the following: The tops of the **gills,** which are exposed where you have cut into the **gill cavity,** are seen on either side. The **heart,** which may be still beating, lies between these on the mid-line in a cavity known as the **pericardium.** It is soft and spongy in its consistency and you should be able to distinguish the paired openings, **ostia,** which lie upon its dorsal surface. How many are there? The **gastric mill** or **gizzard** lies well to the front and is roughly triangular. Note its thin and delicate walls and the two transverse bars of harder material, by which its walls are strenghtened. When the specimen is intact muscles pass from each of these bars, or **sclerites,** to the inner face of the dorsal skeleton. Find the remains of these muscles still attached to the shell which you removed, and also to the posterior sclerites. If the carapace has not been removed too far forward you should be able to see the muscles arising from the anterior sclerite and attached to the inner face of the shell just behind and between the eyes. These muscles form a part of the complex system by which the grinding of the gastric mill is brought about. Passing through the pericardium are large muscles which diverge as they pass forward. If these pull on their forward ends as the fixed point, what movement will they bring about in the abdomen? You can answer this if you understand how the segments of the abdomen are articulated to one another. For this see specimens macerated in caustic potash. The appearance of the region between the heart and gizzard differs with the sex and sexual maturity of the specimen. In a female, with well developed **ovaries,** these latter organs are seen as a bi-lobed mass in front and a median mass behind the heart. In a male the **testes** are less conspicuous, but have the same general "Y" shape. In specimens which are immature or which have recently shed their eggs or sperms the organs are quite inconspicuous and need not be noted for the present. The **digestive gland** which is of a yellowish green color in a freshly killed specimen will be easily made out, but in specimens with large ovaries it may be crowed almost out of sight and only found by pressing aside the latter organs.

(b) Cut off the tops of the gills, sever the extensor muscles of the abdomen at the level of the heart, cut back along either side of the abdomen as far as the telson and remove the dorsal skeleton of this region. The **abdominal extensors** will be found as two thin bands of muscle lying close under the skeleton. They may be taken off with the skeleton, though you should be careful not to tear away anything else. The **intestine** will now be seen in the abdominal region along the mid-line. Beneath and to the sides of the intestine are masses of muscle, which by their combined action flex the abdomen. Compare the bulk of these flexors with that of the extensors. Why should there be such a difference in the size and hence the power of these muscles? Lying on top of the intestine you will perhaps make out a very small transparent thread, the **dorsal abdominal blood vessel.** At the anterior end of the abdomen the median portion of the reproductive organs may be found or, if these are immature, the posterior ends of the digestive glands.

(c) Make an outline (x 2 or 3) of the cephalothorax and abdomen. Put into this the organs as they now lie in place.

(d) Remove the heart and look for ostia on its ventral surface. Note the "Y" shape of the reproductive organs and find their **ducts** leading to the external openings before noted. Remove the reproductive organs, being careful not to injure the digestive gland or the intestine. Trim off more of the gills and pull away the portions of the abdominal extensors which remain in the thorax. Make out the connection of the gizzard with the intestine and the antero-posterior extent of the digestive glands. Cut in from one side and find the **œsophagus;** It is very short and can be best located by noting again the position of the mouth. Trace the intestine to its posterior end, cut off close to the anus and carefully free it up to its union with the gizzard, also free the digestive glands. Cut across the œsophagus and remove the entire digestive tract and its appended glands in one piece. Float out in water, and cut off the left digestive gland close to the tract. Note the region between the gastric mill and the intestine. Open the gizzard along the ventral mid-line, find the teeth, work them together and see how they grind.

(e) Draw a side view from the left, showing the tract and the right gland in position and the place where the left one opens into the tract.

Exercise 5. The Nervous System

(f) Carefully remove all the muscles and viscera from the abdomen. The **ventral nerve cord** will then be seen lying on the mid-ventral line. Notice the **ganglia.** How many do you count? Notice the **lateral nerves.** How are these arranged with reference to the ganglia? In the cephalothorax, the nerve cord is concealed beneath transverse ridges of the ventral wall of the shell. Cut these with heavy scissors and expose the nerve cord, beginning at the hinder end of the cephalothorax and working forward. How many **thoracic ganglia** do you find? Just back of the œsophagus is the large **sub-œsophageal ganglion,** which is connected with the brain by two **connectives** passing around the œsophagus. The **brain** or **supra-œsophageal ganglion** is just behind the eyes. Find the nerves passing from the brain to the eyes and to the two pairs of antennæ. Draw a figure of the nervous system thus exposed, showing accurately the ganglia, the segments in which they lie, and the lateral nerves.

(g) At the anterior end of the body, near the external openings already noted, find the **excretory organs** or **green glands.** The thin **bladder** and underlying **glandular portion** of the organ can be readily distinguished. Refer to textbook for further details. Show the position and shape of these organs by dotted outlines added to the general figure, III (c).

IV. THE APPENDAGES

Exercise 6. Serial Homology and Functional Modification

(a) Examination of the appendages shows that they are obviously modified for a different use in each region of the body. Anteriorly one finds that the appendages have sensory functions. In the region of the mouth they are modified in relation to the siezing and mastication of food. In the middle region they serve for walking legs. In the abdomen the smal-

ler appendages are modified with reference to respiration and reproduction while the last pair, together with the terminal segment or **telson,** constitute the powerful **tail fin.** Comparison of their structure reveals a certain fundamental plan which will be appreciated as study progresses. In the following examination, take the appendages one at a time from the **right** side of the animal and **arrange each in such a way that when completed all your figures will have the same orientation,** according to the more detailed instructions which follow. This is very important for the correct understanding of the homologies between the various appendages. It is also important that the parts of each appendage drawn be completely labeled and that the smaller ones be drawn on a generous scale.

(b) There are all told 19 pairs of appendages. Beginning with the abdomen, count the number of pairs in this region of the body and compare them with the number of segments. The last pair of these is called the **uropods** (tail-feet), the others the **pleopods** or **swimmerets.** Note again the differences in the two anterior pairs of abdominal appendages in the sexes. Remove the right appendage of the fourth abdominal somite by cutting close to the body. A basal piece, the **protopodite,** bears two terminal pieces, an inner **endopodite** and an outer **exopodite.** However markedly any of the other appendages may seem to differ from this plan of structure, all can be shown to be derived from this fundamental plan. The only exception is found in the case of the first antennæ. Draw the above appendage with the end of attachment upward, the exopod to the right and the endopod to the left. Use this same orientation in all your other drawings of appendages.

(c) Remove and draw the uropod of the right side. Orient and label as above.

(d) The thorax has eight pairs of appendages as follows: Four pairs of walking legs or **pereiopods** (walking feet), the great claws or **chelæ**; and three pairs farther forward which will be examined presently. Remove the right fourth pereiopod and the right chela, being sure to get all of the parts of which each is composed. In the pereiopod the two proximal

parts represent a divided protopod, while the remaining five are divisions of the endopod. In the embryo an exopod is present. The great claws resemble the two anterior pairs of pereiopods save for the union of two of the divisions. Can you find where this has occurred? Note the simple modification by which the nipper is formed on the chela. Draw this pereiopod in the same orientation as your previous figures and show by a dotted outline the position the exopod would have if present.

(e) In front of the great claws are three pairs of appendages, known as the **maxillipeds** (jaw feet). The most posterior pair, **third maxillipeds,** are large and easily recognizable. Before removal, the right hand member of this pair should be compared part by part with the walking leg just examined. It has the same parts except that an exopod is present. At one point two of the segments have fused to form a single one as in the chela. This third maxilliped is a very important appendage from the fact that it still has the fundamental plan, and so can be compared with the simpler abdominal appendages; while the structure of its endopod shows how we may interpret the adult structure of the walking leg. Draw this appendage oriented as above.

(f) Examine, without removing, the **second maxillipeds** which lie in front of the third. They will be found to have parts similar to the latter. They should be removed with care not to destroy the **first maxillipeds** which lie close in front of them. Identify, as before without removing, the parts of these first maxillipeds. There is a large **epipodite** which lies in the gill chamber just behind the bailer. Protruding toward the mid-line are two thin flaps which are outgrowths from the protopod, and at about right angles to these are two other projections which are the exopod and endopod. Which is which? Remove the right one and draw on a large scale, orienting as above.

(g) In front of the first maxillipeds are two pairs of **maxillæ,** the parts of which should all be identified before the attempt is made to remove either one. The posterior or **second maxillæ,** have a four-cleft protopodite, a delicate endopod and

an exopod which is fused with the epipodite so that it looks of the fused exopod and epipodite? Before this appendage is like a forward continuation of the latter. What is the function removed the parts of the **first maxilla** should be identified. This, the smallest of all the appendages, consists of three parts, the endopod and a bi-lobed protopod. Which is which? Remove the right second maxilla and draw, orienting as above.

(h) Remove and make a similar figure of the right first maxilla.

(i) The **mandibles** will now be exposed. Against their posterior surfaces are a pair of lobes which are not true appendages. Each mandible consists of a heavy basal portion, on the median side of which is located the cutting edge, which is shown by the embryology to be a development of the protopod and which is comparable to the more delicate median outgrowths on the first and second maxillæ. The three-jointed **palp** which protrudes from the heavy basal piece has its proximal joint formed from the protopod and the other two from the endopod. The exopod is wanting in the adult. Where would it be if it were present? Remove and draw the mandible of the right side orienting as above.

(j) The **second antennæ** will show, when examined in place on the specimen, the typical exopod, endopod and protopod, and the opening of the green glands. Remove the right one of this pair, orient, and draw.

(k) The **first antennæ,** or **antennules** as they are sometimes called, are the only ones which do not show a real division into the three fundamental parts, although their two terminal portions at once suggest the endo- and exopod. Remove one and examine more carefully the region of the **lithocyst** or organ of equilibration.

THE FRESH-WATER MUSSEL

PHYLUM, MOLLUSCA. CLASS, LAMELLIBRANCHIATA

I. THE SHELL

Exercise 1. Genera and Species

(a) The fresh-water mussels or clams are represented by many genera and by species which are numbered in the hundreds. The scientific and popular names of a few of the species common in the Mississippi Valley are as follows:

Quadrula ebena, the nigger head.
Q. pustulosa, the warty back.
Q. metanevra, the maple leaf.
Lampsilis ligamentina, the mucket.
L. anodontoides, the yellow back.
L. rectus, the black sand shell.
Symphynota complanata, the hatchet back.

The directions here given are sufficiently generalized for use with any of these species or with other species which are likely to be found in large numbers.

(b) The shells of the species listed above, or better a more extensive collection of mussel shells, may be used to illustrate the difference between a **genus** and a **species** and between the species of a genus. If such a collection is available, examine the shells of a single genus, noting their peculiarities and the features they all appear to have in common. Examine one or more other genera in the same manner and compare with the first. The genera **Quadrula** and **Lampsilis** are useful for this purpose. By such comparison one can obtain a better idea of what is meant by a genus and a species.

Exercise 2. Structural Features

(c) Examine a pair of shells. In life the right and left halves or **valves** are firmly united by a **hinge.** The outer sur-

face of the shell shows **lines of growth,** produced by the alternation of growing and non-growing periods. In general, these lines mark annual increments. What is the oldest part of the shell judging by these lines? This part is called the **umbo.** Why are the shells commonly eroded at the umbos? Examine the edge of a broken shell to find the layers: **periostracum,** on the outside; **prismatic layer**; and the **mother-of-pearl** within. Can you find lines of growth which give a clue to the manner in which the shell grows in thickness? The prismatic layer may be further demonstrated in a ground section.

(d) The orientation of the animal with reference to its shell is as follows: The valves are "rights" and "lefts". The hinge marks the dorsal, the gape the ventral side. A line drawn through the umbo, at right angles to the long axis of the shell, divides the shell into distinctly unequal parts. The smaller of these parts is anterior. To test your understanding of this orientation, fit the valves together and hold in such a position as to give the same general orientation as the present position of your own body.

(e) Inside the shell are **teeth** which lock tightly when the valves are closed. What is their function? Good sized scars indicate the places of insertion of the **adductor muscles** which bring the valves together. Which is the **anterior** and which the **posterior** adductor? Since there are no muscles which pull the valves apart, how is the opening of the shell accomplished? Shells especially prepared to demonstrate this will be needed.

(f) The scars marking the insertions of the foot-muscles are smaller but easily recognizable. The **posterior retractor** scar lies above the posterior adductor; the **anterior retractor** scar lies posterior to the anterior adductor; the **protractor** scar lies a short distance below that of the anterior retractor. Extending between the adductors and parallel to the margin of the shell, is a line marking the attachment of the retractor muscles of the mantle. The **mantle line** is the so-called "water line" often seen on fresh-water pearl buttons. What can you make out at the edge of the shell regarding the three layers above noted?

(g) Draw (x 1) the outer surface of the right valve, with its dorsal margin toward top of page, and below this the inner surface of the left valve in the same orientation. In beginning the figures, place the right valve on the paper and trace the two outlines. Show all the points of the foregoing section which can be represented.

(h) Determine the effect of acid upon the substance composing the shell, also the effect of a strong alkali like caustic potash. Of what is the shell composed? Why are the heavy-shelled muscles abundant only in regions of limestone rock?

II. THE LIVING MUSSEL

Exercise 3. General Behavior

(a) The living mussels may be studied in large aquaria or better in individual dishes having enough sand on the bottom to allow the clams to bury themselves readily. **Lampsilis subrostrata**, a small pond and slough mussel, is admirable for this purpose and may be studied in a finger bowl. Place an active individual on its side and watch it begin burrowing. The fleshy organ which can be protruded from between the antero-ventral margins of the shells is the **foot.** How does the animal make its way down into the sand and move about? The fleshy membrane exposed between the slightly gaping valves is the **mantle.** In a specimen which lies undisturbed on its side or in one which is embedded in the sand, can you see openings between the right and left sides of the mantle at the posterior end of the animal? Are there **papillæ** along these openings or elsewhere along the mantle margin? Touch parts of this region very gently with a needle and determine its sensitiveness. Can you distinguish any difference in the degree of sensitiveness between the region of these two openings, or **siphons,** and the part of the mantle near the foot? In a quiet specimen, with the siphons well open, watch for currents of water in and out by way of the siphons. The existence of currents may be demonstrated by dropping powdered carmine into the water near the siphons or there may be enough silt

in the water to show the movement. There is a constant, though gentle, current in one siphon and out the other. Which is the **inhalent** and which the **exhalent** siphon? When the mantle edge is strongly stimulated with a needle, note how the shells quickly close driving water out through both siphons. By examining a number of mussels, which have been left undisturbed for some days in an aquarium containing sand, determine the positions normally assumed by the animal in its life upon the bottom. The food of the mussel consists of microscopic organisms which live on or near the bottom. These water currents are of great importance to the animal, because they constitute an essential feature not only, of the food getting but also of the respiration and excretion. When the internal structure has been examined, it will be seen that the animal acts like a sieve, straining out the water and retaining the microscopic food particles. If the dish can be placed in direct sunlight, allow the animal to expand and then test its sensitiveness to light by passing a strong shadow over the siphons. Such points in the foregoing as can be well described in writing should be properly incorporated in your laboratory book.

III. THE EXTERNAL STRUCTURE OF THE BODY

Exercise 4. The Outer Surface of the Mantle

(a) A specimen preserved in formalin or one just killed should be used. As the removal of the shell is not easy for a beginner, you should have special instructions for this preliminary step. Remove the **right** valve and study the mussel from this right side as it lies in the other valve. This places the specimen in the same orientation as the drawings of the shell. Note the soft membrane, the mantle, which conforms to the inner surface of the shell. There will be no breaks in the mantle unless it has been mutilated. Find the ends of the following muscles, the scars of which have already been seen upon the shell: **anterior** and **posterior adductors**; **anterior** and **posterior retractors**; and the **protractor**. Find the line

on the mantle which corresponds to the **mantle line** on the shell. The following internal organs can be more or less definitely recognized, according to the species or the method by which the specimen has been prepared: **digestive gland**; **kidney**; **Keber's organ**; and **pericardium**, containing the heart. By consulting a chart or blackboard diagram, understand their position even if you are not able to locate them at this time in your own specimen. Make a figure of the mussel (x 1) as it thus lies in the left valve of its shell and seen from the right. The removed valve may be wiped dry and the first outline of the drawing made by tracing around it.

Exercise 5. The Organs of the Mantle Cavity

(b) The space enclosed between the right and left halves of the mantle, and in which the **foot** lies, is the **mantle cavity.** Without tearing or cutting, find how the **incurrent siphon** communicates with this. Lift up the mantle edge and examine the foot and the four plate-like **gills**, which extend from the sides and top of the foot to the region of the siphons. Look carefully and see the line along which the outer surface of the outer gill and the inner surface of the mantle meet. Find the **palps**, a pair of leaf-like organs on either side of the foot posterior to the anterior adductor muscles; and note their attachment to the inner surface of the mantle. Remove now the right half of the mantle by cutting from the middle of the incurrent siphon along a line about one-fourth inch below and parallel to the place where the gills and inner face of the mantle meet. At the region of the palps care should be taken to leave these organs intact. Continue the cut just below the anterior adductor muscle and thus expose these organs of the mantle cavity. Trim off to a neat outline the cut edge of the mantle, without injuring gills or palps. Beginning with an outline made by tracing with the right valve, construct a figure to show all of the above organs, omitting for the present the structure of the region above the cut edge of the mantle and the outline of the cut edge itself.

Exercise 6. The Structure and Functions of the Gills

(c) The structure and functions of the respiratory organs may now be studied. By looking into the uninjured ex-current siphon, the **intestine,** ending in the **anus,** will be seen on the posterior face of the adductor muscle. Extending beneath this adductor anteriorly, is a cavity into which a bristle may be thrust for a considerable distance. Being careful not to cut too deep, make an incision at the top of the outer gill near the middle of its length, and expose a cavity running along the top of this gill. By gently probing with the bristle, explore this cavity forward and back. How does it end in either direction? With the bristle thrust in as a guide, cut in either direction and expose this **supra-branchial** cavity from the anterior end of the gill to its opening into the **cloaca,** as the region just within the excurrent siphon is called. Trim away the tissue on each side of the cut so that the whole diameter of this supra-branchial cavity can be seen. The upper cut edge should be made to pass along the lower margin of the posterior adductor and end above the anus; the lower cut edge should pass out to where the two siphonal openings meet. The floor of this cavity is cut by a series of transverse partitions, **inter-lamellar junctions,** separating cavities, the **water-tubes** of the gill. If the gill is not too much shrunken, you can pass a bristle down any of these water-tubes to the ventral edge of the gill where it ends blindly. With scissors, remove a piece of the outer gill and cut sections at right angles to these water-tubes. Examine under water with a hand lens. The water-tubes and inter-lamellar junctions will be seen cut transversely. It is in these water-tubes that the young of the clam begin their development and you may find the gill distended with embryos. By looking forward from beneath the posterior adductor and gently exploring with a bristle, you can see that the inner gill of this side and the two gills on the other have each a supra-branchial cavity and water-tubes, as in the gill just examined. Make a diagram of this system of cavities as it would appear if seen from the dorsal view.

(d) You should now understand how the water current,

from which the clam obtains its food and oxygen and by which its carbon dioxide and other wastes are carried out, flows through the gills. Coming in by way of the incurrent siphon, the water bathes the organs of the mantle cavity. From here it passes through microscopic openings, the **ostia,** which lead from both inner and outer surfaces of all the gills, into the water-tubes. Passing upwards in the water-tubes, it emerges in the supra-branchial cavities; and passing backward in these, it reaches the outside through the cloaca and exhalent siphon. The water which passes out has been strained of such micro-organisms as are too large to pass through the ostia of the gills. These organisms upon coming in contact with the surfaces of the gills, foot or mantle are entangled in a slimy secretion, **mucus;** and carried by cilia along definite lines which converge toward the palps. Passing between the inner and outer palp of either side, the food is at length delivered to the **mouth,** a slit-like opening which lies below the anterior adductor and between the continuation of the inner and outer palps.

(e) Examine under the microscope a bit of tissue cut from the gill of a living clam and one from the mantle and make out the cilia on these surfaces. The ostia will be demonstrated in stained sections of the gill, taken in the same plane as the rough sections previously made with scissors. Place carmine or pieces of cork upon the gills or upon various parts of the mantle surface of a living specimen and note the result. Compare with the action of the cilia in mouth of the frog. Add to your previous drawing these details of the supra-branchial and cloacal cavities, taking care to show the cut edges where they occur. Put in arrows to show the course of the water currents. Why are we justified in speaking of the mantle cavity as a part of the external surface of the animal, and of the gills and foot as "external" organs?

IV. THE INTERNAL STRUCTURE

Exercise 7. The Heart and Pericardium

(a) In the region above the gills and in front of the posterior adductor is a cavity, the **pericardium.** Make a small

incision in its wall and lifting with forceps cut away the wall, exposing the **heart,** which consists of a single median **ventricle,** wrapped about the **intestine;** and of delicate right and left **auricles,** which lead from the sides of the pericardial cavity. These last can be better seen if the dissection is placed under water. The dark colored **kidney** will be seen underlying the pericardium. Add to your previous figure the pericardium as thus exposed and its contained organs. Show clearly the cut edge of its wall.

Exercise 8. The Kidneys and Reproductive Organs

(b) Lift up the outer gill and remove by cutting away the inner wall of its supra-branchial cavity. Locate the supra-branchial cavity of the inner gill and cut into this to expose its full length. The dark color of the kidney will probably show through the median wall of this latter cavity. Well toward the anterior end, there will be found against this dark area two small openings. They are not as easy to find in some species as in others but can usually be located. The dorsal one of these openings is the **external opening of the kidney,** the ventral is the opening of the reproductive gland or **genital pore.** On the other side of the foot are similar openings for the left side of the body. Note that the kidney thus opens into a cavity from which the water flows immediately to the outside. The eggs upon leaving the genital pore are fertilized by sperm which have been shed to the outside water by an individual of the opposite sex and entered the supra-branchial cavity of the female. After being fertilized the eggs pass into the water-tubes and there develop as far as the larval stage, known as the **glochidium.**

(c) Beginning at the anal end, dissect the intestine away from its union with the upper surface of the posterior adductor muscle. Turn the specimen up on the ventral edge of its shell, and look into the pericardium from above. By taking hold of the intestine and turning it over anteriorly, expose the extreme anterior end of the pericardium. Careful probing with a fine headed bristle should reveal the opening from the

pericardium into the kidney. Push the bristle posteriorly as far as it will go and thrust another finely tipped bristle through the external opening of the kidney pushing it back in the posterior direction. Cutting into the substance of the kidney will now reveal the fact that one bristle lies in an upper and thin-walled, the other in a dark colored and thick-walled cavity. Near the posterior adductor these upper and lower limbs of the kidney unite.

Exercise 9. The Foot and Visceral Mass

Using the handle of a scalpel, scrape the tissue of the kidney away from the top of the foot. Note the two **posterior retractors,** the ends of which have been previously observed. Still using the scalpel handle, free the posterior retractors from the adductor muscle, and cut the left one where it attaches to the shell. Can you see how they retract the foot? Locate the **anterior retractors,** and the **protractors** of the foot. How do they function? Leaving the intestine intact and attached to the foot and **visceral mass** (the term applied to the softer and less muscular part of the body which lies above the foot), continue using the handle of the scalpel, and break away the remaining attachment of the visceral mass to the shell, being careful not to injure the mouth. Remove this part of the body from the shell leaving the anterior adductor behind.

Exercise 10. The Digestive System

(d) Remove the palps and any remains of mantle or gills from each side of the visceral mass. Using a sharp scalpel, split this mass and the foot as nearly into right and left halves as possible, leaving the free end of the intestine attached to the left half. Examine the cut surface of the left half under water and pinned down in a pan. The visceral mass is composed of a pasty substance, made up largely of the reproductive gland, in which coils of the digestive tract are embedded. Follow any of these coils which are cut by the section. The flattened **œsophagus** leads upwards to an enlargement, the **stomach,** into which the right and left halves

of the **digestive gland** open. The course of the tract should be examined in textbook or chart figures.

Exercise 11. The Nervous System

(e) Look on the ventral surface of the posterior adductor muscle for a yellowish body which is the fused right and left **visceral ganglia** of the nervous system. Look for nerves extending out from this. The paired **cerebral ganglia** will be seen upon the ventro-posterior face of the anterior adductor muscle. In the soft tissue just above the upper margin of the foot and a little distance below the mouth, you will find by gentle scraping, if they are not already exposed, the pair of **pedal ganglia.** They are yellowish in color and of firmer texture than the tissue in which they are embedded. Nerves will be found running out from them. Show in your general drawing the position of these three pairs of ganglia. Understand, from lectures, textbook or charts, how the three pairs of ganglia are united by paired connectives, and what parts of the body their nerves supply.

V. THE LIFE-CYCLE OF THE MUSSEL

Exercise 12. The Glochidium

(a) At certain periods of the year the gills of the mussel function as brood-pouches and are found distended with the developing eggs or with the larval stage known as the **Glochidium.** Examine in a watchglass of water some of these glochidia just removed from a freshly opened mussel. Note the two halves of the **shell,** the **adductor muscle** between them, and certain fine projections, the **sensory** hairs, on the inner surface. Are there **hooks** at any point on the shell? Beyond the valves of the shell none of the organs of the adult are visible. Watch the glochidia for any movements and record nature of same. The egg is fertilized and develops within the parent as far as this larval stage which is sooner or later shed from the mussel, by way of the out-going water current. After reach-

ing the bottom the glochidia must come in contact with the fins or gills of a fish and fasten themselves there in order to continue their development. After leaving the fish they have developed all the organs of the adult in miniature and can begin life on the bottom. Draw the glochidium on a large scale, as it appears gaping open, and also from a lateral view when closed. The closure of all the specimens can be easily effected by adding a few drops of alcohol or of methyl green.

Exercise 13. The Infection of Fish with Glochidia

(b) Take a considerable number of living glochidia in a finger bowl of clean water and put into this two small fish. Watch how and where the glochidia attach. If the fish do not keep the water sufficiently agitated to prevent the glochidia settling to the bottom, it must be stirred gently. After five or ten minutes take one of the fish and put into an aquarium. Kill the other, without pressing upon the gills or fins, and pin it ventral side up in a pan of water. Remove gills, fins, and tail, and examine them with microscope in a watchglass. How and where are the glochidia attached? Draw one or more glochidia, attached to a gill or fin, and on such a scale as to make the larvæ about one-half inch in diameter. Make an estimate of how many glochidia there are on this one fish; of how many were produced by the single mussel.

(c) The fish, which was placed in the aquarium after infection, should be examined at the next laboratory period and the condition of the glochidia with reference to the tissue of the fish determined. Or fish infected 24 to 48 hours previously may be provided for examination on the same day as the foregoing. Record the condition of these glochidia and if you have time make figures. The glochidia of different mussels parasitize different fish. To carry any glochidium through its parasitism, it is necessary to infect a fish that will carry that particular glochidium. The pond mussel **Lampsilis subrostrata** and the sunperch **Lepomis pallidus** furnish such a combination. In the laboratory, the glochidia remain on the perch for a period of from 10 to 30 days, according to the temperature,

and then drop to the bottom as young mussels which crawl actively about. They may then be seen with the eye and collected without difficulty.

VI. SPECIAL STUDIES

Exercise 14. Transverse Sections

(a) For a review of many points brought out by the foregoing study, the examination of sections, cut through a specimen from which the shell has been removed, is valuable. In sections from the region of the heart, which are perhaps more instructive than any others, the following structures should be made out and compared with the conceptions and figures already obtained; **mantle**; **foot**; **gills**; **supra-branchial, mantle** and **pericardial cavities**; **kidney**; **Keber's organ**; **ventricle**; **auricles**; and **intestine.** How is the shell related to the whole? How does the water pass from the mantle cavity to the supra-branchial cavities? Where are the water-tubes? Draw such a section on a large scale, labelling all the parts and showing the course of the water by arrows.

Exercise 15. Further Dissection of the Nervous and Digestive Systems

(b) A second specimen may be used for a review of the structures previously dissected and the more complete demonstration of the nervous and digestive systems. Remove the shell and mantle, examining again any points not previously clear. Locate the **cerebral** and **visceral ganglia,** without cutting anything but the mantle. By careful dissection, follow one of the two nerves, which may be seen leading anteriorly from the visceral ganglia, to its union with the cerebral ganglia. From these latter, three pairs of nerves arise: the pair of **cerebro-visceral connectives,** just dissected; a pair of **mantle nerves**; and the **cerebro-pedal connectives.** Follow one of the last to its union with the **pedal ganglia.** Determine the number of nerves arising from the latter. Make a figure of the entire nervous system.

(c) This same specimen may be used for a dissection of the digestive tract. To do this, remove the entire animal (including the two adductors) from its shell and pin out under water. Begin at the mouth and dissect out the tract to show, **œsophagus, stomach,** openings of **digestive glands,** and coils of the **intestine.** These parts must be exposed by removing the side of the visceral mass which is uppermost and following each part of the intestine as it is found. Make a figure of the entire digestive tract.